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THE COÖRDINATION OF THE VENTRICLES.

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ALL who have studied the mammalian heart in action have been impressed by the admirable synchronism of the contractions of the two ventricles. So perfectly do the right and left hearts work together that the observer is led at once to the opinion that their unison must be enforced by some master mechanism, such as a group of ganglion cells. The presence of nerve cells in the heart, and especially their presence in the basal part of the ventricles, lends color to this view, and we cannot therefore be surprised that the coördination of the thousands of muscle fibres of the ventricle into one harmonious contraction, and especially the synchronism of the contractions of the two ventricles, differing as they do in their muscular power and in the work required of them, should be believed to be a function of the nerve cells to which so great a share in the regulation of the heart-beat is ascribed.

I have demonstrated in a previous investigation[1] that at any rate the coördinated contraction of a single ventricle is not dependent on the influence of nerve cells. It was found that the mammalian ventricle might be kept beating for a very long time if supplied with blood through a cannula tied into the coronary artery. Ventricles cut entirely away from the auricles and thereby for the first time completely isolated, were kept contracting thus. Even any portion severed from the remainder will beat when fed with blood through the artery supplying it. The inevitable conclusion from these experiments is that *the coördination mechanism, whatever it may*

[1] PORTER: Journal of experimental medicine, 1897, ii, p. 301.

be, is present in all parts of the ventricle. The same method was then used to determine whether the coördination of the ventricle was dependent on nerve cells. A cannula was tied into the ramus descendens of the left coronary artery not far from the apex of the left ventricle; and that part of the apex of the ventricle which could be fed through the cannula was excised and perfused with blood. In a few moments regular and strong contractions set in. An hour and forty minutes later the apex was still beating, when the experiment was broken off. In another experiment the apical ten millimetres of a dog's ventricle was kept in coördinated rhythmical contraction during several hours. The contractions thus secured were not the rhythmic response of the ventricle to an abnormal constant stimulus due to changes in the perfusion blood or to the 0.8 per cent sodium chloride solution with which the blood was frequently diluted; the contractions are usually more vigorous on undiluted blood than on that containing normal saline solution. They occur also when the piece of apex is fed in the living animal with the wholly normal blood supplied to the rest of the heart. Thus a branch of the ramus descendens in the apical region on the anterior surface of the left ventricle was freed from its bed and an oval piece of the ventricular wall supplied by this branch was separated from the remainder of the ventricle by an incision that ran parallel to the surface of the heart about four millimetres beneath the pericardium. The isolated piece remained in connection with the ventricle only by a strip of pericardium several millimetres long, which supported the artery and its accompanying veins, and was absolutely free of muscular tissue; long-continued, spontaneous, coördinated contractions were obtained from this piece, the rate being somewhat slower than that of the whole heart. As the apical portion of the ventricle contains no nerve cells,[1] these experiments furnish conclusive proof that nerve cells are not essential to the coördinated contractions of the ventricle.

The problem of the dependence of the coördination of a single ventricle upon nerve cells having been thus solved, the next question to be attacked is whether the remarkable synchronism of the right and left ventricle is also independent of nerve cells.

I was at first in doubt whether the apical halves of both mammalian ventricles could be made to beat together, because the arterial supply is such that both ventricles cannot be fed very well through a cannula

[1] This fact has been recently once more demonstrated by the careful work of SCHWARTZ: Archiv für mikroskopische Anatomie. 1898. lviii. p. 63.

in the ramus descendens of the left coronary artery, which is the only thoroughly practicable branch to the apex. My doubts were entirely dispelled by the following experiments.

Experiment November 14, 1898. A dog anæsthetized with morphia and ether was bled from the left carotid artery, the blood defibrinated, and filtered through glass wool. Meanwhile, warm 0.8 per cent sodium chloride was allowed to flow into the right jugular vein. After a short interval the dog was bled again from the carotid artery, and the blood defibrinated as before. The heart was now taken out, a cannula tied in the ramus descendens at the beginning of the apical half of its course, and the apical half of the heart severed from the basal half with a sharp amputating knife. The apex of the heart is formed chiefly by the left ventricle, so that the transverse incision just described includes strictly speaking the apical half of the left ventricle, but only the apical fourth of the right. Fibrillation set in as soon as the section was made, but probably disappeared before perfusion was began; for when the cannula was connected with a reservoir containing the defibrinated blood at a temperature of 36° C. and under a pressure of about 100 mm. Hg., strong, fully coördinated contractions of the whole preparation began. The whole mass beat together as one piece; the synchronism of the right and left halves was perfect. Fifteen minutes later, the gas flame under the water tank surrounding the blood-reservoir having been left too high, the temperature of the blood rose to nearly 40° C., and the heart began to fibrillate. The supply of blood was then interrupted, in the hope that asphyxia would cause the fibrillation to disappear, as is often the case. Asphyxia failing, recourse was had to cold normal saline solution.[1] On reducing the temperature of the heart, the fibrillation gave place to coördinated contractions. When the warm blood was suddenly turned on again, the preparation at once shortened visibly and began to fibrillate.[2]

[1] PORTER: This journal, 1898, i, p. 71.

[2] During the past two years I have repeatedly observed that the tonus of the ventricle was apparently greatly increased at the onset of fibrillation. The same fact has long been noted by students of the effect of electrical stimulation on the heart. Repeated experiments on the isolated ventricle have shown me that this shortening is an invariable, or almost invariable accompaniment of fibrillation, and that coördinated beats do not return unless the extreme, apparently tonic contraction is considerably lessened. It is evident that during fibrillation not all of the contractile units are in action at the same time or in the same way. Either the continued shortening observed is (1) a true tonus, dependent perhaps on the excessive action of contractile substance of the sluggish sort (Grützner), or (2) it is due to an abnormal, long-continued, tonic contraction of a number of the cardiac muscle fibres. The appearance of such a spasm at several points might break up the normal conduction of the contraction wave by interposing here and there regions, the intense spasmodic contraction of which would block the passage of the contraction wave, just as it is blocked experimentally by

Experiment November 15, 1898. A preparation of the apical half of the ventricles of a dog's heart was made as described in the preceding experiment. On perfusion the heart at once began to beat very strongly. The coördination of the right and left side was perfect.

It may be remarked that the contractions thus obtained are much more vigorous than those given by smaller pieces of the ventricle. The force of the beat is indeed astonishing. With each stroke blood shoots from the margins of the strip a considerable distance into the surrounding air. No better method for demonstrating the compression of the intramural vessels in systole could be desired.[1]

The experiment, just cited, together with similar ones on November 16 and 17, demonstrate conclusively that *the synchronism of the mammalian ventricles is not dependent on nerve cells.*

Another problem now arose. Is the coördination of the ventricular contractions in warm-blooded animals accomplished by the muscular elements alone or by the aid of the nerve fibres with which all parts of the heart are so plentifully supplied? Engelmann's well-known experiment, slitting the frog's ventricle by a zigzag cut running to and fro across the line taken by the nerves in such a way that the nerves entering the upper end of the zigzag strip are in all probability severed by one of the cuts before reaching the end of the strip, has answered for the batrachian heart in favor of the muscular fibres. A like experiment can be done on the mammalian heart, and with the same result. Contrary to long-established belief the mammalian ventricle perfused with blood diluted with sodium chloride solution, or wholly undiluted, as a rule continues to beat in spite of extensive incisions. In my paper "On the Cause of the Heart-beat,"[2] it is recorded that on March 22, 1897, the isolated heart of a cat was made to resume its regular contractions by feeding the coronary arteries through the aorta with defibrinated cat's blood. While the heart was

squeezing the muscle fibres together with a clamp, and would leave the remaining muscle fibres dissociated and in confusion. The second of the two above explanations is the more probable, in my judgment, but the evidence which I have collected, while more than sufficient to justify the making of an hypothesis, is not yet wholly decisive. Dr. C. C. Stewart has suggested to me that the shortening may be explained by the evident failure of all the muscle fibres to contract together: the contraction of some of the very numerous fibres must almost necessarily though by chance coincide, and this continuous coincidence would give the appearance of continuous, tonic contraction.

[1] PORTER: This journal, 1898, i, p. 145.

[2] PORTER: Journal of experimental medicine, 1897, ii, p. 392.

in full play the right ventricle was separated from the left and the interventricular septum cut out. Both right and left ventricles continued their rhythmic contractions. Two days thereafter, a cat's heart was removed from the body and fed with cat's blood diluted with 0.8 per cent sodium chloride solution. Coördinated contractions were secured. The right ventricle was then almost wholly separated from the left, yet both ventricles continued to beat in unison. The interventricular septum was now extirpated, but the synchronism of the ventricles was not disturbed. On slitting each ventricular portion from apex to base, the incisions being carried almost to the auricles, the four pieces beat synchronously. Similar incisions made in the frog's heart have been recently described in the interesting communication of Professor von Vintschgau.[1] The mammalian heart, however, is better adapted to these studies than the heart of the frog because of its large size, and especially because the nutrition of the frog's ventricle is suspended by opening the ventricle, while the nutrition of the mammalian ventricle may be carried on through the coronary arteries without the aid of the ventricle.

Persuaded by the observations of 1897 that the mammalian ventricle would continue to beat even under the conditions of Engelmann's procedure, the following experiments were performed.

Experiment November 16, 1898. The apical half of a dog's heart was perfused with blood through a cannula in the ramus descendens, as described above. Admirable coördinated contractions synchronous over both ventricles were secured. The ventricles were slit from the apex towards the base into many pieces, each supplied by a branch of the descendens artery. All contracted synchronously. The junction of each severed piece with the rim of uncut muscle at the base of the preparation was narrowed by transverse cuts until only a small neck remained, so that each piece communicated with the other pieces only by this narrow bridge. The muscular tissue remaining in the bridge was less than a square millimetre in cross section. Coördinated contractions were still synchronous in all the pieces. Some of the bridges were divided, leaving these pieces hanging from the basal ring of the preparation only by the nutrient artery and its connective tissue. Each piece thus separated beat with a rhythm of its own and no longer contracted synchronously with any other portion. Zigzag cuts were now made in several strips, but in each case they failed to prevent synchronous contraction; so long as a slight muscular connection was preserved the contractions of the partially severed portions remained synchronous.

[1] Von Vintschgau: Archiv f. d. ges. Physiol., 1898, lxxiii, p. 381.

Experiment November 17, 1898. As in yesterday's experiment, good coordinated contractions were obtained in the apical half of the dog's heart, and the ventricles slit from the apex toward the basal portion of the preparation into long pieces, each of which contained a branch of the coronary artery. One of these long strips was now cut into three parts by three pairs of incisions; one cut of each pair was carried from the left margin of the strip nearly to the nutrient artery, the other at the same level, from the right margin nearly to the artery; the three portions were thus left united by the nutrient artery, connective tissue, and a few shreds of muscular tissue. All three beat in unison. The muscular tissue joining the uppermost to the middle piece of the three was now divided. Immediately the middle and the lowest piece, thus deprived of all muscular connection with the remainder of the ventricle, began to beat with a rhythm of their own, but still in unison with each other. The uppermost piece on the contrary continued to contract synchronously with the remainder of the heart.

When one piece of several connected together merely by the nutrient artery and a very small muscular bridge was thrown into fibrillation by rapidly repeated induction shocks, all the pieces at once fibrillated (in other hearts, this did not always succeed, probably because the power of conduction in the bridge was too much reduced), but when adjoining pieces were connected only by the artery and connective tissue and not by muscle fibres fibrillation was no longer transmitted from one to the other.

Engelmann's zigzag incisions were also tried on this heart, with complete success; synchronous contractions were observed in all the parts, weakened indeed by the interference with the blood-supply but still unmistakably present.

The fact that an extraordinarily small muscular connection between two pieces of mammalian ventricle will suffice to maintain the synchronism of the two was confirmed by numerous trials on fifteen additional animals, three dogs and twelve cats. There can be no doubt that the mammalian and the frog heart are alike in that a very small bridge of cardiac tissue suffices to carry the excitation wave from one part of the heart to the neighboring parts. It is in the highest degree improbable that in all of the numerous instances cited here the heart nerves should take the extraordinary recurrent and roundabout course necessary for them to reach all parts of the zigzag preparation; the course of these nerves has in fact been frequently described — they do not present such extraordinary aberrations. Unless we take refuge in unbridled speculation and surmise without real histological evidence that the cardiac nerves form a continuous net-work, there is little escape from the conclusion that the

conduction in these experiments is not by any means of nerves. It is therefore highly probable that *the synchronism of the mammalian ventricles is maintained through muscular and not through nervous connections.*

Having come thus far it is necessary to reflect on the part the auricle may play in the coordination of the ventricular contractions. The auricles are connected with the ventricles by both muscular and nervous tissue. It is through the auricle that the excitation wave reaches the ventricle, and through the auricle also pass impulses that modify the character of the ventricular contraction. What more probable than that the auricles, or the sinus venosus operating through the auricle, should have a hand in the coordination of the ventricles? If the right ventricle be separated entirely from the left but the connections of both ventricles with the auricle allowed to remain undisturbed, will not the synchronism of the ventricles be preserved?

The first experiment in this series was made November 17, 1898. The experiments began with an attempt to coordinate portions of one ventricle through their connection with the auricles. Typical protocols are as follows.

Experiment November 23, 1898. A dog was bled in the manner already described, the heart excised, and a cannula tied into the circumflex branch of the left coronary artery. The part supplied by this artery, embracing nearly the whole of the left ventricle, was now separated from the remainder of the ventricles by incisions made near the anterior and posterior margins of the interventricular septum respectively. Care was taken not to injure the auricle. On supplying the separated part with blood through the nutrient artery, it began to beat in the usual manner. The auricle also contracted, but the contractions were feeble, and were not always followed by contractions of the ventricle. The ventricle was now divided into several strips by incisions running from the apex nearly to the auricle, between the principal branches of the ramus circumflexus, so that each strip continued to receive blood from a branch of the artery. All the strips continued to beat synchronously. One of the strips was now entirely separated from the rest of the ventricle by continuing the incisions which bounded the strip on its two sides through the auriculo-ventricular groove. The circumflex artery was carefully avoided, so that the nutrition of the strip now isolated from the ventricles was preserved. The broad connection of the strip with the auricle was left untouched. Upon the last muscular connection with the ventricle being severed, the rate of beat of the isolated part suddenly changed. It no longer contracted synchronously with the rest of the ventricle but at a slower rate.

Similar experiments were repeated many times in the three dogs and ten cats which served for this portion of the investigation. The result has invariably been the same. Any part of either ventricle can be separated almost entirely from the contiguous portions without interfering with the synchronism of the whole; the division can be completed on one side by an incision crossing the auriculo-ventricular groove without disturbing the synchronism; the remaining side can be reduced to a very small muscular bridge, and the parts will still beat in unison; but so soon as the bridge is destroyed, the synchronism is destroyed, although both portions of the ventricle possess their normal connections with the auricle. Very rarely — only once in my numerous experiments — a few synchronous beats in the two portions of ventricle may indeed be witnessed, but the observer will readily perceive that these are chance coincidences of two separate and variable rhythms. Experiments of this sort therefore give a negative reply to the question whether the ventricles can be coördinated through the auricles.

The procedure just described is open to the objection that the auricle and ventricle as a rule did not beat in their normal sequence in these experiments, indicating perhaps that the conduction across the auriculo-ventricular groove was more or less at fault. Thus the failure of the auricle to coördinate the ventricles might possibly be due to a disturbance of conduction between auricle and ventricle.[1] The method should maintain the normal sequence of contraction. Only when the isolation of a portion of the ventricle does not disturb the normal sequence between the auricles and the remainder of the ventricle can we be sure that the independent beat of the separated part is not caused by a general failure in conduction from auricle to ventricle. The objection is valid; we cannot be sure; though the probability of error, considering the number of observations, is not great. The method should be improved.

The necessary improvement in the method was secured in the following way.

Experiment December 3, 1898. A cat anæsthetized with ether was bled from the left carotid artery, but not to excess, and the blood added to that collected from two other cats. The animal was now tracheotomized, artificial respir-

[1] The difficulty here mentioned is not due to a reduction of vitality by the excessive bleeding of the animal before the experiment; in all the later experiments the greater part of the blood was obtained from one animal and the heart to be perfused was taken from a fresh animal, from which very little blood was drawn.

ation begun, the chest opened, the left carotid ligated, and a cannula tied into the innominate artery. The right coronary artery was laid bare near its origin. The cat was then placed on the inclined support described in Miss Hyde's paper in this Journal,[1] and the cannula connected with the reservoir containing defibrinated blood[2] at a temperature of about 33 C. and a pressure of about 80 mm. Hg. The aorta was then ligated just distal to the origin of the left carotid artery, and the right ventricle opened near the apex. On opening the stop-cock between the feeding bottle and the innominate artery, the blood passed from the aorta through the coronary vessels to the right heart and thence into the chest cavity, gradually filling the deep pocket formed by the diaphragm and the lower part of the thorax. From this reservoir it was removed from time to time, filtered, and replaced in the feeding bottle. The action of the heart was thoroughly satisfactory; all parts contracted vigorously and each contraction of the auricles was followed by a contraction of the ventricles.

The right ventricle was now slit up, parallel with the anterior interventricular furrow, the section passing beneath the exposed right coronary artery completely across the auriculo-ventricular groove. The synchronism of the ventricles and the normal auriculo-ventricular sequence was not altered by this. A second incision was made near to and parallel with the posterior interventricular furrow almost to the auricle. All parts continued to beat as before. The section was extended across the bridge of ventricular substance and the auriculo-ventricular groove. The right ventricle, thus wholly isolated from the left, immediately began to beat with a slower rhythm. The remainder of the heart continued at the old rate, each auricular contraction being followed by a ventricular contraction.

Obviously in this experiment there can be no question of the preservation of the normal power of conduction; the ample natural connection of the isolated portion of the right ventricle with the auricles was left untouched; the remainder of the ventricles beat in sequence with the auricles; it cannot be supposed that conduction to the isolated portion was instantaneously lost in consequence of the mechanical injury of an operation repeatedly shown not to entail such consequences; the sections made in this and previous experiments never interfered with coordination or sequence unless they completed the separation of one portion of the ventricle from the rest of the ventricular mass; it may be mentioned, also, that had the effects observed been due to mechanical injury, they should have shown

[1] Miss I. H. Hyde: This journal, 1898, i, p. 215.

[2] The blood contained some 0.8 per cent sodium chloride solution, but it may be remarked once more that undiluted blood will serve, though less convenient.

themselves on both sides of the incision instead of only on the side separated; the cause of the alteration in the separated portion cannot, then, be mechanical injury. The experiments demonstrate that any portion of the ventricle will beat synchronously [1] with any other portion, so long as the two are connected by muscle tissue, but that synchronism immediately fails when the muscle bridge is broken, in spite of the fact that both portions may retain their normal connection with the uninjured auricles. Further than this it would be unsafe to venture. The facts in our possession do not permit a positive statement as to the nature of the coördination impulse, and it is undesirable to add another speculation to the mass with which the physiology of the heart is cumbered.

On the same day two other instructive experiments of this kind were performed. In the first of these the isolated portion of the right ventricle beat with the same frequency as the remainder of the heart, but with a different rhythm; the beats were not synchronous with the other portion of the ventricles. In the last of the three experiments, the isolated portion of the right ventricle continued to beat as if nothing had happened; the synchronism of the two portions of the ventricles was unchanged. The exception, however, proved the rule, for post mortem it was found that the first incision — that made along the left anterior border of the right ventricle — had not entirely severed the tissues near the auriculo-ventricular groove; a few muscular fibres still connected the apparently isolated portion with the rest of the ventricle.

It is true that the result here recorded is in some degree a negative one. Yet the number and character of the experiments seem to leave no room for doubt, and I do not therefore hesitate to conclude that *the synchronism of the ventricular contractions is not a function of the auricles but is managed by the ventricles themselves.*

Summary.

1. The synchronism of the mammalian ventricles is not dependent on nerve cells.

2. The synchronism of the mammalian ventricles is probably maintained through muscular and not through nervous connections.

3. The synchronism of the ventricular contractions is not a function of the auricles but is managed by the ventricles themselves.

[1] It is of course understood that time is required for the excitation to pass from one part of the ventricle to another, — the synchronism is relative, not absolute.

ON THE PATHS OF ABSORPTION FOR PROTEIDS.

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THE text-books of physiology agree, at present, in assigning to the portal circulation the task of transporting away from the alimentary canal ingested proteids after they have been modified by the natural digestive processes. The experimental evidence in favor of this view is familiar, and has scarcely been questioned; recently, however, Asher and Barbèra[1] have published the results of an experiment which leads them to conclude that the thoracic duct forms — though perhaps only to a small extent — a channel for the transportation of digested proteids to the blood. These investigators experimented upon a large dog having a well-healed gastric fistula. A cannula was introduced into the thoracic duct of the animal after it had fasted for sixty hours. During continued narcosis the hunger-lymph was collected for an hour; two hundred grams of dry albumin (from blood) were then introduced into the stomach through the fistula and thereupon the lymph collected for six hours in hourly portions. Total solids, ash, and total nitrogen were determined in the seven portions, and the proteid content of the lymph was calculated from these data. The results obtained may be summarized as follows:

Experiment of Asher and Barbera.

AVERAGE COMPOSITION OF LYMPH, PER HOUR.

		Before Proteid feeding.	*After Proteid feeding.*
Lymph,	grams	16.50	27.91
Total Solids,	grams	1.15	2.18
" "	per cent	7.05	7.81
Ash,	grams	0.08	0.10
"	per cent	0.49	0.35
Nitrogen,	grams	0.132	0.251
"	per cent	0.809	0.899
Proteid (N × 6.25),	grams	0.825	1.572
"	per cent	5.056	5.632

[1] ASHER and BARBÈRA: Centralblatt f. Physiologie, 1897, xi, p. 403. "Der Brustgang betheiligt sich also nach Eiweissnahrung an der Fortfuhrung des Eiweisses in das Blut, wenn auch in geringer Menge." (*Ibid.*, pp. 406-7.)

In connection with the data obtained, Asher and Barbèra point out that the curves plotted to indicate the changes in the composition of the lymph from hour to hour show a striking similarity to those obtained for the hourly nitrogen excretion in the urine after meals;[1] and the authors are inclined to attach significance to the correspondence between the curves.

In support of earlier experiments[2] demonstrating that — in man — the absorption of ingested proteids is not ordinarily a function of the lymphatics, I. Munk[3] has published a brief review of the experiment of Asher and Barbèra. He points out that the total proteid found in the lymph during the six hours of the absorption period exceeds the proteid in the hunger-lymph, calculated for an equal period, by only 4.485 grams, *i. e.*,

Total proteid in "absorption" lymph	=	9.435 grams
" " " "hunger" " (6×0.825)	=	4.95 "
Proteid presumably absorbed into lymphatics	=	4.485 "

Of the two hundred grams of proteid introduced into the dog's stomach, one hundred and thirty grams were recovered at the end of the experiment. Accordingly, since seventy grams of the albumin introduced had disappeared from the alimentary tract, the additional quantity of proteid found in the lymph during six hours of absorption corresponds to only 6.4 per cent of the proteid absorbed. In other words, 93.6 per cent of the material absorbed presumably passed into the circulation by channels other than those leading to the thoracic duct. Munk further points out that these results were obtained with excessive quantities of proteid: two hundred grams being given to the dog at one time.

I have repeated the experiment of Asher and Barbèra with modifications in two respects. First, a smaller quantity of proteid was fed; and secondly, the proteid employed was a readily soluble one — Witte's "pepton" — which previous experience had shown to be satisfactorily utilized by dogs.[4] A gastric fistula had been made on the 14-kilo animal several months previously. The wound was

[1] Cf. TSCHLENOFF: Correspondenzblatt f. Schweitzer Aerzte, 1896, p. 1; VERAGUTH: Journal of physiology, 1897, xxi, p. 112; HOPKINS and HOPE: *ibid.*, 1898, xxiii, p. 271; HOPKINS: Schaefer's Text-book of physiology, 1898, i, p. 585.

[2] MUNK and ROSENSTEIN: Virchow's Arch. f. d. exper. Pathologie, 1891, cxxiii, p. 496.

[3] I. MUNK: Centralblatt für Physiologie, 1897, xi, p. 585.

[4] Cf. MENDEL and JACKSON: This journal, 1898, ii, pp. 10–11.

perfectly healed and the dog in good health.[1] After forty-eight hours fasting a glass cannula was tied into the thoracic duct during morphine narcosis (0.16 gram morphine sulphate + 0.016 gram atropine sulphate subcutaneously), a little chloroform-ether being administered when necessary. The dog remained in narcosis during the experiment. The portion of lymph collected during the first ten minutes after introduction of the cannula was not retained, since owing to the operative procedure the flow of lymph is at first always somewhat more rapid than subsequently.[2] The "hunger" lymph was collected during an hour; thereupon fifty-five grams of Witte's "pepton" containing 14.0 per cent nitrogen were introduced with 125 cb.cm. water through the fistula into the stomach. The lymph was then collected in hourly portions during the six succeeding hours. The animal was killed by bleeding and the stomach contents removed and examined. The intestine was found empty; the appearance of the gastric and intestinal mucosa was normal. The stomach contained 205 cb.cm. fluid with mucous flocks in suspension. The filtered gastric contents, composed largely of primary proteoses with a little true peptone, furnished the following data on analysis:

Total solids, ...	30.23 grams
Total nitrogen (Kjeldahl), ...	4.42 "
Nitrogen in total solids, ...	14.6 per cent
Total acidity (equivalent to HCl), ...	0.77 "

No *free* HCl present.

The lymph portions were analyzed in the customary manner, nitrogen being determined by the Kjeldahl method and the proteid calculated from the figures thus obtained. The results are summarized in the accompanying table.

The fifty-five grams of Witte's "pepton" introduced into the stomach contained 7.70 grams of nitrogen. At the end of the experiment unabsorbed proteid containing 4.42 grams nitrogen was recovered from the stomach. The nitrogen in the "pepton" absorbed thus amounted to $7.70 - 4.42 = 3.28$ grams, equivalent to 23.4 grams of Witte's "pepton." Nearly one half the proteid fed had thus been absorbed; in Asher and Barbèra's experiment about one third of the albumin disappeared. In my experiment the total nitrogen in the

[1] The form of gastric cannula used has been described in This journal, 1898, i, p. 191.

[2] Cf. HEIDENHAIN: Arch. f. d. ges. Physiol., 1891, xlix, p. 215.

TABLE OF ANALYSES OF LYMPH.

Time.	Amount Collected.	Total Solids.		Ash.		Nitrogen.		Proteid (N. × 6.25).	
hours	grams	grams	per cent	grams	per cent	grams	per cent	grams	per cent
1. (Before feeding)	13.46	0.551	4.09	0.122	0.90	0.0609	0.45	0.381	2.83
2. (After feeding)	16.59	.625	3.83	.147	.88	.0674	.40	.421	2.53
3. " "	11.50	.472	4.10	.104	.90	.0475	.41	.297	2.58
4. " "	12.64	.548	4.33	.105	.83	.0614	.48	.384	3.03
5. " "	8.79	.393	4.47	.068	.77	.0454	.51	.284	3.23
6. " "	14.60	.674	4.61	.125	.85	.0768	.52	.480	3.28
7. " "	15.90	.708	4.45	.128	.80	.0757	.47	.473	2.97
Six fasting hrs. (calculated) .	80.76	3.306	4.09	0.732	0.90	0.3654	0.45	2.286	2.83
Six absorption hrs. (2.—7.) . . .	80.02	3.420	4.27	.677	.84	.3742	.46	2.339	2.92

lymph during the absorption period amounted to 0.374 gram; the nitrogen-content of the starvation lymph (1.), calculated for an equal period, would amount to $6 \times 0.0609 = 0.365$ gram. The difference between these figures — 0.0088 gram N. = 0.053 gram proteid — which may be attributed to the proteid absorbed, is insignificantly small in amount, and is far smaller than the corresponding figure obtained by Asher and Barbèra with a considerably larger quantity of proteid. Neither the total quantity of lymph collected nor the percentage of proteid present was increased during the six hours of absorption.

With reference to a possible correspondence between lymph flow and urea excretion after proteid absorption it may be remarked that — according to Tschlenoff — if peptone is ingested instead of ordinary proteids, the maximum urea excretion is reached in the second hour. To emphasize the comparison — as Asher and Barbèra have done — between the lymph flow and the urea excretion observed in quite different experiments seems to me of doubtful value; for the lymph flow is readily influenced by purely mechanical factors. Thus it is quite possible that in my experiment movements of defecation observed in the dog in the 6th hour may explain the increased flow

of lymph unaccompanied by any alteration in the percentage of proteids; just as slight pressure on the abdomen will immediately cause an increase in the flow.

Asher and Barbèra[1] have formulated the theory that the lymph is a product of metabolism in the glands of the body, and they have attempted to demonstrate that the lymph flow increases in all cases where there is accelerated glandular activity. In view of the work accomplished by the alimentary epithelium and the liver during the digestion and absorption of proteids, the new theory demands that an increased flow of lymph should result after proteid feeding. The present experiment with "pepton" failed to yield results in conformity with this view; it might, however, be said by the defenders of the theory that no pronounced increase in lymph flow is to be expected during the absorption of soluble and readily diffusible products like Witte's "pepton," since the work of the intestines is comparatively slight under these conditions. Such, at least, is the explanation which they have applied to the absence of accelerated lymph flow in carbohydrate nutrition.

The results of the absorption experiment with moderate quantities of a soluble proteid fail to modify the current statements regarding the paths by which proteids are absorbed; *i. e.*, under ordinary circumstances by far the greater share in the process must still be delegated to the capillaries of the villi.

[1] Asher and Barbèra: Zeitschr. f. Biologie, 1898, xxxvi, p. 210; Asher: IV International Physiological Congress; Centralbl. f. Physiol., 1898, xii, p. 486.

A CHEMICO-PHYSIOLOGICAL STUDY OF CERTAIN DERIVATIVES OF THE PROTEIDS.

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THE marked activity which has characterized the study of the proteids and their primary digestion or cleavage products during the last decade has been especially manifest in two distinct directions. In the first place, effort has been directed toward a study of the various transitions which these substances undergo in the several phases of digestion and nutrition, with a view to obtaining clearer insight into the genetic relationship of the various chemical products and incidentally into their chemical nature. In the second place, much attention has been given to a study of the physiological action of the more characteristic products of gastric and pancreatic proteolysis, and more especially the behavior of these products when introduced into the system through channels other than the gastro-intestinal tract. This latter phase of physiological research has been carried out in the majority of cases with a mixed product resulting from the digestion of blood-fibrin with gastric juice, known as "Witte's

Pepton," and composed of a variable proportion of proteoses with some true peptone.

It is needless to refer here in detail to the extensive literature which these latter investigations have created. It will suffice to recall the fact that the more important observations on the effect of so-called "peptones" when introduced directly into the blood current were made by Schmidt-Mülheim[1] and Fano[2] in Ludwig's laboratory. The results they obtained have in part become classic, and supplemented by certain later observations made by other investigators may be briefly summarized as follows: The primary products of digestion (proteoses) when introduced directly into the circulation of dogs in doses from three to five decigrams per kilogram of body-weight produce a rapid and marked fall of arterial blood pressure, which slowly returns to the normal. If after the injection of the proteoses blood be withdrawn from an artery, it fails to clot as rapidly as under normal conditions, or may even remain liquid indefinitely. The intensity of the effects produced depends in part on the rapidity with which the substance is introduced into the circulation and to a lesser degree on the quantity of material employed. Further, when the effects of an injection have disappeared a second similar injection may fail to produce the characteristic action, a certain degree of immunity having been established. Coincident with these results there usually occurs a very marked increase in the flow of lymph in the thoracic duct, together with a decreased coagulability of this fluid. Other changes in the urinary secretion and respiration are less constant, and have been less thoroughly investigated.

These statements will, we believe, receive general acceptance as expressing well-established truths. There remain, however, a large number of problems to the solution of some of which the experimental work of this paper is directed. Thus, the questions arise: Are the various physiological results — fall of arterial pressure, loss of blood coagulability, lymphagogic influence, etc. — genetically related or independent phenomena? Is the influence exercised directly upon the blood or indirectly through specific organs or tissues? Are all organisms equally susceptible to these actions? Are the effects produced common to all decomposition or cleavage products of proteids? What light do the phenomena throw upon normal or abnormal processes in the body?

[1] Schmidt-Mülheim: Archiv f. Physiol., 1880, p. 30.

[2] Fano: *Ibid.*, 1881, p. 277.

Such are some of the problems presented. Recent work by a number of investigators, however, has thrown light in many directions and revealed a multitude of facts which promise advance in this particular field. Still, few investigators have attempted to use for study more characteristic or more definite chemical products than the earlier workers had at their command, or where this has been attempted there has in some cases at least existed a certain degree of indefiniteness concerning the exact nature of the substances employed. This is the more to be regretted as there are many facts which tend to show that the primary cleavage products of the proteids may differ from each other in their chemical constitution, and if so one might naturally look for pronounced variations in physiological behavior. Further, it unfortunately happens that previous experimental data obtained with isolated proteoses and peptones are frequently deficient or entirely wanting on many points of interest. We therefore present our results obtained with various types of proteid cleavage products in the hope that they may contribute somewhat to a broader and more concise knowledge of the subject. The several products we have made use of are fully described as regards mode of preparation, composition, and chemical characters in another section of this paper. Their physiological action is described in the following pages.

The Physiological Effects of Some Cleavage Products of the Proteids when introduced Directly into the Circulation.

Technique. — Our experiments were carried out exclusively on dogs. The animals were made to fast for at least twenty-four hours previous to the operative procedure; they were narcotized by a subcutaneous injection of morphine mixed with atropine, and a mixture of chloroform and ether was administered during the operative interference and at other times when necessary. Arterial pressure was recorded by a mercurial manometer connected with the carotid, or femoral artery, and the injections were made into one of the facial veins from a burette attached to a glass cannula. Intervals of one second were recorded beside the blood pressure curve by means of a metronome time-marker. In observing the rate at which the blood coagulated, portions were drawn from the right femoral artery. The cannula was removed after each withdrawal of blood and thoroughly cleansed, and the blood was allowed to escape for a second or two before the

next portion was taken. The samples of 2 c.c. each were received in graduated cylinders with a calibre of one centimetre. The "rate of coagulation" was taken to be the time which elapsed before the cylinder could be inverted without the loss of a drop.

The elimination of the substances through the kidneys, as well as the rate of flow of the urine, was studied by collecting the urine from cannulæ introduced directly into the ureters. In those experiments in which the lymph was collected and analyzed, the directions of Heidenhain[1] were followed.

The proteoses, peptones, etc., studied were usually dissolved in 0.7 per cent sodium chloride solution. With substances insoluble in dilute saline fluids, such as heteroalbumose and antialbumid, it was found best to use a few drops of ammonium hydroxide to facilitate solution, any excess of ammonia being driven off by heat. The volume of fluid injected was ordinarily 4 c.c. per kilo of body-weight up to a maximum of 50 c.c. The doses of the different substances employed were varied in order to ascertain their relative activity. The injections were accomplished as rapidly as possible, and seldom occupied more than thirty seconds. Indeed, many of the tracings show a slight initial rise of blood pressure due probably to the increased volume of the blood reaching the heart, before the fall caused by the substances injected.

Specific substances studied. — In selecting substances for study we have been influenced by the fact that the evidence already at hand is sufficient for establishing the general action of mixed proteoses and peptones. It has therefore seemed to us desirable to study especially the physiological action of certain well-defined cleavage products with a view to obtaining evidence of the action of the individual bodies present in the ordinary digestion products of the proteids. Thus, an investigation of the physiological action of the immediate precursors of antipeptone has seemed especially desirable in view of the discordant observations of previous investigators with this product and also in view of the peculiar significance which is attached to this substance through recent chemico-physiological research.[2] Furthermore, we have been impressed with the importance of having true antipeptone for experiment, and consequently, we have employed pure antialbumid as the mother substance from which to prepare the antipeptone. Following is a list of the substances studied; all of

[1] Heidenhain: Archiv f. d. ges. Physiol., 1891, xlix, p. 209.

[2] Siegfried et al.

which were prepared with great care according to the methods detailed in the second part of this paper.

Antialbumid (A), formed from coagulated egg-albumin by the action of dilute sulphuric acid at 100° C., and purified by digestion with pepsin-acid, etc.

Antialbumoses (B), formed by the digestion of the above antialbumid with gastric juice.

Antialbumoses (C), formed by the digestion of the above antialbumid with pancreatic juice.

Antipeptone (D), formed by the digestion of antialbumid with pancreatic juice.

Antialbumid (E), a more insoluble form, separating from an alkaline solution of the above antialbumid when subjected to pancreatic digestion.

Antialbumid (F), prepared from crystallized edestin by the action of dilute sulphuric acid.

Hemialbumoses (G), formed from coagulated egg-albumin by dilute sulphuric acid.

Heteroalbumose (H), formed in a similar manner.

Hemipeptone (I), formed by the same method as the hemialbumoses.

Protogelatose (J), formed from pure gelatin by gastric digestion.

Deuterogelatose (K), formed by pancreatic digestion.

Gelatin-peptone (L), formed by pancreatic digestion.

Albumose-like body (M), formed from coagulated egg-albumin by the action of dilute sulphuric acid at 100° C.

Influence on blood pressure. — The first more comprehensive and systematic study of the physiological action of proteoses and peptone individually was made by Grosjean.[1] This investigator prepared the several products from blood-fibrin by artificial gastric digestion, using the methods of Kühne and Chittenden in isolating and purifying the mixed proteoses and peptone. Two products only were prepared: *i. e.*, "propeptone," a varying mixture of primary and secondary proteoses, and "peptone" or amphopeptone. These products were introduced intravenously into the circulation of dogs and other animals, and arterial blood pressure registered. Grosjean concluded from his experiments that "propeptone" introduced into the circulation causes a considerable fall of arterial pressure, the depression occurring at once after the injection and quickly reaching its maximum. Further, the extent to which arterial pressure is lowered is apparently not influenced by the size of the dose, except when the latter amounts to less than 15 centigrams per kilo of body-weight, in which case the

[1] GROSJEAN: Archives de biologie, 1892, xii. This paper contains references to the earlier literature.

depression is much less marked. Moreover, as a rule, the pressure once lowered is very slow in returning to the normal. Thus, in one experiment where 1 gram of "propeptone" per kilo was injected, arterial pressure was still below the normal three and a half hours after the injection. With peptone, there is likewise produced a fall of arterial pressure which, however, is less marked, slower in reaching its maximum, and of shorter duration than that caused by like doses of "propeptone." Further, when the pressure returns toward the normal it frequently rises slightly above the original height, after which it again falls, although this second depression is slight as compared with the first.

Arthus and Huber[1] working with gelatoses and caseoses formed by pancreatic digestion, observed that when these bodies were introduced intravenously into dogs in large doses (2 grams gelatoses per kilo; 1.5 grams caseoses per kilo), and the injections carried out rapidly, there was a marked lowering of blood pressure comparable to that obtained with the fibrin proteoses. Here, too, no attempt was made to differentiate the mixed caseoses or gelatoses into more specific products.

In a recent paper from this laboratory[2] attention was called to the action of deuteroalbumose and peptone obtained by the proteolysis of egg-albumin with papain. The experiments showed an immediate lowering of blood pressure when these products, in doses of 0.5 gram per kilo of body-weight, were injected into the circulation, the pressure, however, soon returning to the normal.

Thompson[3] has investigated the specific action of Witte's "pepton" on the vasomotor system, and more recently this study has been extended[4] to include the action of pure peptone, antipeptone, and deuteroalbumose, although the details of these latter experiments have not yet been published. The results obtained agree in a general way with those already recorded by Grosjean. They indicate, however, that smaller doses of these products than has generally been supposed, suffice to produce physiological effects. It is furthermore definitely shown that lowering of arterial pressure is due to a vascular

[1] Arthus and Huber: Archives de physiologie, 1896, p. 857.

[2] Chittenden, Mendel, and McDermott: This journal, 1898, i, p. 255.

[3] Thompson: Journal of physiology, 1896, xx, p. 455.

[4] Thompson: Interim report of a committee consisting of Professors Schafer, Sherrington, Boyce, and Thompson. Report by the Secretary of the British Association for the Advancement of Science, 1896-97.

dilatation as the result of a peripheral or local action on the blood vessels. No central nervous influences could be demonstrated. This vasomotor action is not confined to the splanchnic area, but is common to all the blood vessels, and is exercised presumably upon the endings of the vasomotor nerves and on the muscles of the blood vessels, — the decreased irritability of the neuro-muscular apparatus making the latter incapable of responding to the normal vaso-constrictor impulses. In addition, it is to be noted that Thompson observed that deuteroproteose produced a far more profound and enduring influence on arterial pressure than pure peptone, but less so than the same dose of Witte's "pepton." Hence, deuteroproteose cannot be regarded as the most potent constituent of Witte's "pepton;" a statement which agrees with Pollitzer's[1] observations. Lastly, in agreement with other workers (Pollitzer, Spiro, and Ellinger[2]) Thompson found that antipeptone contrasts very noticeably with the other products of proteid digestion studied in its action on blood pressure. The fall of pressure produced was *temporary* at most.

Our experiments on blood pressure, the results of which are summarized in the following table, indicate that antialbumid, a product whose physiological action has not heretofore been investigated, agrees with the antipeptone derived from it in producing comparatively slight effects on mean arterial pressure. In like doses, however, antialbumid is somewhat more vigorous in action than antipeptone. Further, it is evident that antipeptone derived from antialbumid produces essentially the same results as antipeptone formed by the direct action of pancreatic juice on a natural proteid. Antialbumoses, on the other hand, act most energetically in lowering blood pressure. In no case was a return of mean arterial pressure to the normal observed within an hour after injection of the various albumoses studied. The same was true of the hemialbumoses. The albumoses of different origin act qualitatively and quantitatively alike in great degree, heteroalbumose being apparently most vigorous in its effects. This corresponds with the observations of Pollitzer,[3] and we are inclined to attribute the pronounced effects produced by Witte's "pepton" to the considerable admixture of heteroproteose, which it has repeatedly been shown to contain.[4]

[1] Pollitzer: Journal of physiology, 1886, vii. p. 283.

[2] Spiro and Ellinger: Zeitschr. f. physiol. Chem., 1897, xxiii, p. 135.

[3] Pollitzer: *loc. cit.*

[4] Kühne and Chittenden: Zeitschr. f. Biologie, 1884, xx, p. 15.

TABLE, SHOWING EFFECTS UPON ARTERIAL PRESSURE, MEASURED IN MILLIMETRES OF MERCURY.

Experiment.	Weight of animal, kilos.	Grams per kilo injected.	Nature of the substance employed.	Pressure before injection.	Pressure just after injection.	5 minutes after.	15 minutes after.	30 minutes after.	1 hour after.	1½ hours after.	2 hours after.
I	8.8	0.3	Antialbumoses (B) .	180	90	40	45	50		75	
II	6.2	0.3	" " .	85	45	30	35	50	70		
III	11.3	0.3	Antialbumoses (C) .	140	30	30	30	45	55	95	110
IV	6.0	0.3	" " .	130	37		105	125			
V	18.0	0.3	Hemialbumoses (G) .	170	65	40	38	50	65	85	
VI	7.5	0.18	Heteroalbumose (H) .	160	135	30	20	30	45	45	45
VII	9.8	0.2	" " .	115	75	30		75			
VIII	7.0	0.3	Antialbumid[1] (A) .	80	20	45	90	90			
IX	6.0	0.3	" " .	150	80	40	115	125			
X	5.4	0.3	" " .	130	70	125	150	145	150	135	
XI	7.6	0.14	" " .	150	55	40	115	125	140	150	
XII	14.0	0.15	" " .	140	90	135	130				
XIII	30.0	0.03	" " .	65	30	20	45	60	65		
XIV	8.4	0.17	" " .	140	140	140	140	140			
XV	6.8	0.07	Antialbumid (F) .	95	95	85	110	100			
XVI	9.0	0.3	Antipeptone (D) .	170	160	160	190	170			
XVII	6.5	0.3	" " .	90	40	80	90	90			
XVIII	9.0	0.3	" " .	110	60	135	145	150			
XXXI	4.5	0.45	" " .	120	55	70	140	140			
XIX	11.2	0.3	Hemipeptone (I) .	125	20	120	105			80	
XX	6.5	0.3	" " .	125	60	110	130	130	130		
XXI	13.5	0.5	" " .	130	40	85	130	120	145	145	
XXII	6.4	1.0	" " .	115	50	20	45	60	110		
XXIII	5.0	0.3	Albumose-like body (M)	110	40	20	50	110	110		
XXIV	5.0	1.5	Protogelatose (J) .	140	55	135	150	135			
XXV	4.2	0.3	Gelatin-peptone (L) .	100	75	100	95	100	100		
XXVI	6.0	0.6	" " .	105	40	60	115	120	115		
XXVII	21.0	0.35	Deuterogelatose (K) .	115	115	115	120	120	120		
XXVIII	7.0	0.5	" " .	120	50	95	115	120	120		
XXIX	15.0	0.7	" " .	130	60	120	150	150			
XXX	6.5	1.0	" " .	135	35	65	125	140	120		

[1] All of the samples of antialbumid were dissolved by the aid of a little dilute ammonia except in Experiment XIV, where dilute sodium carbonate was used.

Our experiments with hemipeptone agree with the results obtained by Grosjean and later by Thompson with amphopeptone isolated by Kühne's method. Further, between hemipeptone and antipeptone there is little or no difference of action when like amounts of substance are employed. It is interesting, however, to contrast the action of equal doses of hemipeptone (XIX–XX) with that of the hemialbumoses (V) from the same source. It is only with doses of 1.0 gram of hemipeptone per kilo that a prolonged fall of pressure is

obtainable, and even with this quantity the pressure again resumes its normal within an hour. These facts seemingly suggest more or less pronounced difference in the chemical structure of the proteose and peptone molecules, — a point which we have taken occasion to emphasize in other connections.[1]

The experiments with protogelatose and deuterogelatose in agreement with the observations of Arthus and Huber, fail to show any such marked influence as is obtained with the corresponding albumoses. True gelatin-peptone, on the other hand, approaches hemipeptone and antipeptone in the intensity of its action. The great difference which the albuminoid products show, quantitatively at least, in contrast with the derivatives of the native proteids serves to emphasize the other striking differences between gelatin and true proteids as regards physiological equivalence.[2]

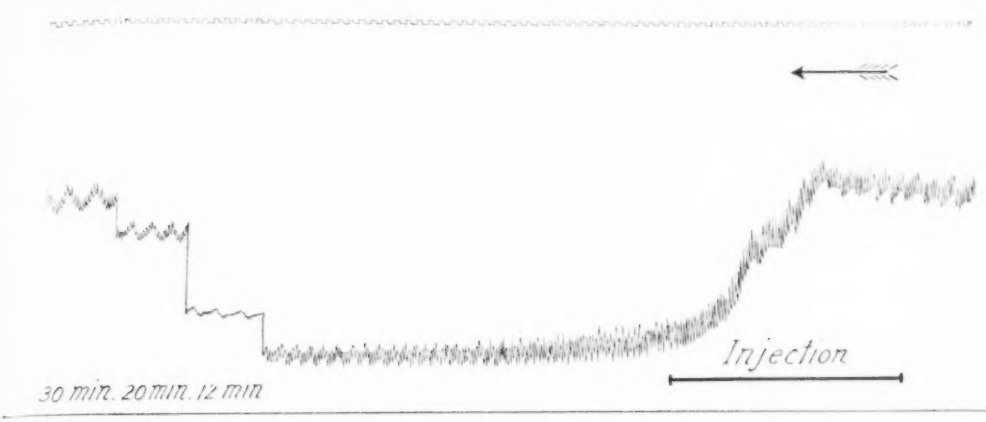
Lastly, attention is directed to the slight initial rise in pressure immediately succeeding the injection of the various substances into the circulation as shown in the tracings. This rise, which is undoubtedly due to the rapid entrance of the fluid into the vascular system,[3] gives evidence of the rapidity with which the injections were carried out. This fact is not without significance, since the variation in effects of intravenous injections of proteoses has been demonstrated (cf. Thompson, p. 119) to be dependent in part on the rapidity of the injections. It seems reasonable therefore to assume that the intensity of vasomotor effects is not so much a function directly of the absolute quantity injected as of the quantity in the circulation per unit of time, or in other words, of the concentration of the substance in the blood.

The accompanying blood pressure tracings have been selected from typical experiments. They were recorded with a mercurial manometer, as already described. The time is recorded in seconds, and the line of zero pressure is likewise recorded. The curves all read from right to left, and only such portions have been reproduced here as furnish evidence of the observations discussed in the preceding pages.

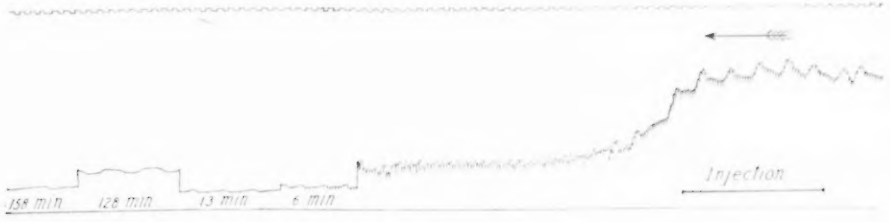
[1] Chittenden and Mendel: Journal of physiology, 1894, xvii, p. 78. Chittenden: Digestive proteolysis, 1894, p. 42. Also recent papers from Hofmeister's laboratory. Zeitschr. f. physiol. Chem., 1897–98.

[2] Cf. I. Munk: Archiv f. d. ges. Physiol., 1894, lviii, p. 309. Also Mendel and Jackson: This journal, 1898, vol. ii, p. 21.

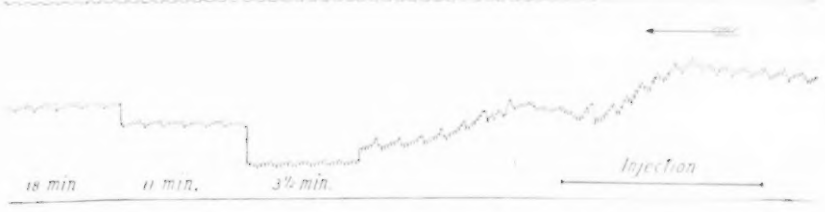
[3] Cf. Thompson: Journal of physiology, 1896, xx, p. 455.



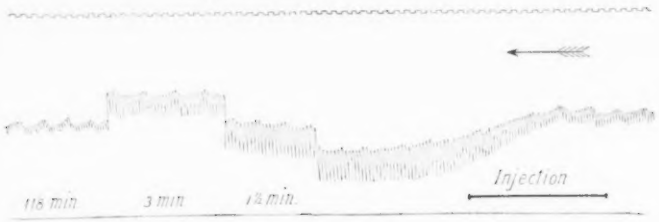
Experiment IV. 6 kilo dog. Injection of ANTIALBUMOSES (C). 0.3 gram per kilo.
One half the original size.



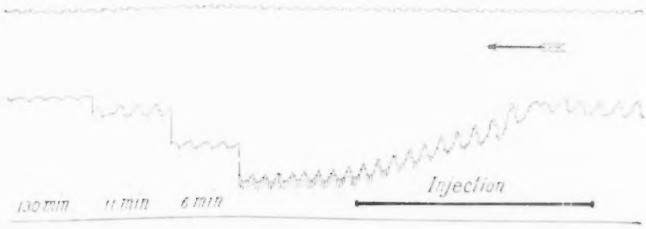
Experiment VI. 7.5 kilo dog. Injection of HETEROALBUMOSE (H). 0.18 gram per kilo.
(Cf. tracing Exper. *XXIX b.*) One fourth the original size.



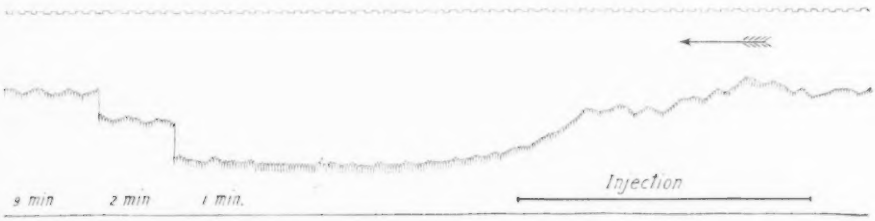
Experiment IX. 6 kilo dog. Injection of ANTIALBUMID (A). 0.2 gram per kilo.
One fourth the original size.



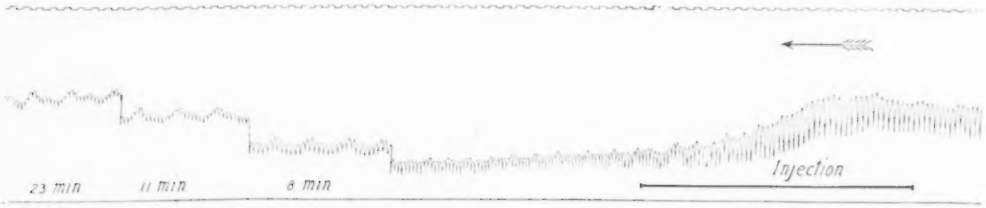
Experiment XVIII. 9 kilo dog. Injection of ANTIPEPTONE (D). 0.3 gram per kilo. One fourth the original size.



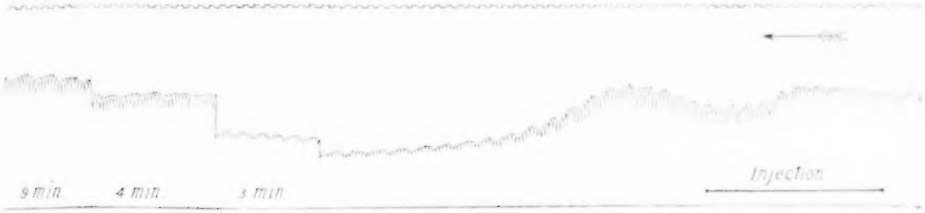
Experiment XXI. 13.5 kilo dog. Cannula in thoracic duct. Injection of HEMIPEPTONE (I). 0.5 gram per kilo. One fourth the original size.



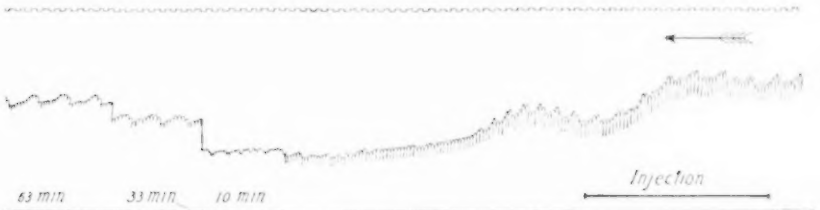
Experiment XXIV. 5 kilo dog. Injection of PROTOGELATOSE (J). 1.5 grams per kilo. One fourth the original size



Experiment XXVI. 6 kilo dog. Injection of GELATINPEPTONE (L). 0.6 gram per kilo. One fourth the original size.



Experiment XXIX a. 15 kilo dog. Injection of DEUTEROGELATOSE (K). 0.7 gram per kilo, preceding injection of heteroalbumose. (Cf. tracing *Exper. XXIX b.*) One fourth the original size.



Experiment XXIX b. 15 kilo dog. Cannula in thoracic duct. Injection of HETEROALBUMOSE (H). 0.1 gram per kilo after previous injection of 10.5 grams deuterogelatose (K). (Cf. tracing of *Exper. IX.*) One fourth the original size.

Influence on blood coagulation. — The older literature on the influence of so-called "peptone" on blood coagulation is too familiar to physiologists to require detailed consideration here. Briefly stated, the researches[1] made have demonstrated (*a*) that "peptone" does not of itself possess any clot-preventing power, *i. e.*, the action is an indirect one; and (*b*) that accordingly the clot-preventing power must be attributed to some substance formed within the organism, and possessing a peculiar influence on the blood. Furthermore, it seems probable that the peculiar substance is not a transformation product of the "peptone" itself, but is rather a result of other changes taking place in the body.[2] More recently, through the researches of

[1] SCHMIDT-MÜLHEIM: Archiv f. Physiol., 1880, p. 30; FANO: *Ibid.*, 1881, p. 277; POLLITZER: Journal of physiology, 1886, vii, p. 283; CAMPBELL: Studies from the Biological Laboratory, Johns Hopkins University, 1887, iv, p. 1; GROSJEAN: Archives de biologie, 1892, xii; LEDOUX: *Ibid.*, 1896, xiv, p. 63.

[2] Cf. CAMPBELL: *Loc. cit.*

Contejean,[1] Gley and Pachon,[2] and especially through the researches of Delezenne,[3] the seat of formation of the anti-coagulating substance has been delegated to the liver, and the important rôle which the leucocytes play in this action has been demonstrated by Delezenne (1898). The explanation formulated at present is as follows: It has long been known that the intravenous injection of "peptone" leads to a pronounced disintegration of leucocytes. Delezenne assumes that this process is accompanied by the liberation of two classes of products, the one accelerating and the other inhibiting blood coagulation. As types of such bodies, the researches of Lilienfeld[4] and others have indicated nucleoproteids, which give rise to intravascular clotting, and histon, a peculiar peptone-like body occurring in leucocytes in the compound nucleo-histon. When these two classes of compounds (clot-retarding and clot-accelerating substances) are formed incidental to the breaking down of the leucocytes, the liver in its protective rôle preserves the organism from destruction through intravascular clotting by combining with the clot-accelerating substance. A condition thus results in which the blood loses completely or partially its power of coagulating on withdrawal from the vessels. The relative rôle of liver and leucocytes is demonstrated by the fact that if a solution of peptone is passed through an excised dog's liver a fluid results which readily produces "peptone" effects when injected into the circulation of a dog. If, however, the liver is previously freed from blood by lavage through the vessels no clot-retarding fluid can be obtained in the manner described. Delezenne's theory therefore implies the existence of two substances antagonistic in their influence on blood coagulation, — a condition which Spiro and Ellinger[5] had previously regarded as probable. These latter investigators observed that the injection of acids or of antipeptone overcomes the clot-retarding power of albumoses, while Dastre and Floresco[6] had previously called attention to the increased alkalinity of "peptone plasma" and the influence of neutralization in inducing

[1] CONTEJEAN: Archives de physiologie, 1895.

[2] GLEY and others: *Ibid.* It is to be noted, however, that Starling (Journal of physiology, 1895, xix, p. 15) has not been able to confirm part of these experiments.

[3] DELEZENNE: Archives de physiologie, 1897, p. 646; 1898, p. 508.

[4] LILIENFELD: Zeitschr. f. physiol. Chem., 1895, xx, p. 89.

[5] SPIRO and ELLINGER: Zeitschr. f. physiol. Chem., 1897, xxiii, p. 121.

[6] DASTRE and FLORESCO: Archives de physiologie, 1897, p. 216. Also FLORESCO: *Ibid.*, 1897, p. 777.

coagulation. Lastly, there is evidence that so-called "peptone" may under some conditions hasten the coagulation of the blood. Thus, Thompson[1] has pointed out that with doses of Witte's "pepton" above 2 centigrams per kilo coagulation is retarded. With doses from 7 milligrams to 2 centigrams per kilo coagulation may be considerably accelerated.

Our experiments on this subject were intended to throw light not so much upon the direct mechanism by which the variations in clotting power are produced, as upon the relative effectiveness of various proteid and albuminoid products. A large number of substances, for the most part extracts of various animal tissues or organs, are known to have the power of retarding blood coagulation either *in vivo* or *in vitro*, or both. Grosjean observed that the proteoses from blood-fibrin retard, or even prevent coagulation indefinitely. This action is not produced *in vitro*.[2] True peptone, he found, never acts as vigorously as the proteoses, and confines its influence to a temporary retardation. In either case the lack of coagulation is very rapidly manifested. Thompson[3] has recorded similar results with purified peptone. Deuteroproteose gave him inconstant results, while antipeptone hastened blood coagulation, in conformity with the observations of Spiro and Ellinger.[4] Arthus and Huber[5] have found that much larger doses of gelatoses (2 grams) and caseoses (1.5 grams) per kilo are necessary than in the case of fibrin-proteoses to bring about an absence of coagulation. Lastly, a few experiments have been reported on the action of the albumoses and peptone formed by papain digestion.[6]

The accompanying table, giving the results of our own experiments, shows the time required for the complete coagulation of 2 c.c. blood withdrawn from the femoral or carotid artery at various intervals, expressed in minutes or hours. The normal clotting-time given is in every case the average of two or more observations previous to the injection of the substance under investigation.

[1] Thompson: Journal of physiology, 1896, xx. p. 455.

[2] Cf. Campbell.

[3] Thompson: Report of Secretary, *loc. cit.*

[4] Spiro and Ellinger: Zeitschr. f. physiol. Chem., 1897, xxiii, p. 121. See also the older experiments of Pollitzer and of Fano with tryptone.

[5] Arthus and Huber: Archives de physiologie, 1896, p. 865.

[6] Chittenden, Mendel, and McDermott: This journal, 1898, i, p. 255.

TABLE SHOWING EFFECTS UPON THE COAGULATION OF THE BLOOD.[1]

Experiment.	Weight of animal; kilos.	Grams per kilo injected.	Nature of the substance employed.	Normal coagulation.	Just after injection.	5 minutes after.	15 minutes after.	30 minutes after.	1 hour after.	1½ hours after.	2 hours after.
I	8.8	0.3	Antialbumoses (B) .	6	8	—	—	—	—	—	—
II	6.2	0.3	" " .	6	16	—	—	20h		20	10
III	11.3	0.3	Antialbumoses (C) .	2	—	—	—	—	—	—	—
IV	6.0	0.3	" " .	12	—	—	—	—	—	—	—
V	18.0	0.3	Hemialbumoses (G) .	2	16h	—	—	—	—	—	—
VI	7.5	0.18	Heteroalbumose (H) .	4	—	—	—	—	—	—	—
VII	9.8	0.2	" " .	4	—	—	—	—	—		
VIII	7.0	0.3	Antialbumid (A) . .	6	6	14h	1½h	1½h	45		
IX	6.0	0.3	" " . .	2	13	16h	—	16h			
X	5.4	0.3	" " . .	6	40	2h	30	30	7		
XI	7.6	0.14	" " . .	5	30	16	—	16h	16h		
XII	14.0	0.15	" " . .	4	57	70	27	17	9		
XIII	30.0	0.03	" " . .	3	9	16	25	45	14		
XIV	8.4	0.17	" " . .	1	6	4	8	13	6	9	8
XV	6.8	0.07	Antialbumid (F) . .	3	4	9	4	5			
XVI	9.0	0.3	Antipeptone (D) . .	3	3	3	2	3	3		
XVII	6.5	0.3	" " . .	5	5	8	10	6	10		
XVIII	9.0	0.3	" " . .	4	4	5	5	4	3		
XXXI	4.5	0.45	" " . .	2	4	3	4	10	6		
XIX	11.2	0.3	Hemipeptone (I) . .	9	1	3	3	4	5		
XX	6.5	0.3	" " . .	4	2	3	4	9	7		
XXI[2]	13.5	0.5	" " . .	3	3	4	6		2		
XXII	6.4	1.0	" " . .	4	4h	—	—	—	—	—	
XXIII	5.0	0.3	Albumose-like body (M)	9		6	35	37	20	14	
XXIV	5.0	1.5	Protogelatose (J) . .	10	4	5	6	4			
XXV	4.2	0.3	Gelatin-peptone (L) .	4	3	2	3	4	5		
XXVI	6.0	0.6	" " .	3	2	2	4	4	3		
XXVII	21.0	0.35	Deuterogelatose (K) .	2	2	2		1			
XXVIII	7.0	0.5	" " .	3	3h	3h		20	3	2	
XXIX[2]	15.0	0.7	" " .	3	4	2	8	2	2	2	
XXX	6.5	1.0	" " .	5	2h	—	—	3h	15	4	

[1] The figures in the table denote the number of minutes before coagulation, except when followed by *h*, when they denote hours. All samples marked — were still fluid after standing 24 hours.

[2] The lack of any noticeable action upon the time of coagulation in these two experiments is due to the fact that the lymph was withdrawn through a cannula in the thoracic duct. See under *lymph-formation* for explanation.

The data presented show (1) That in doses of 3 decigrams per kilo of body-weight the albumoses, both hemi and anti, irrespective of the mode of formation, immediately cause suspension of coagulation for at least 24 hours, even in blood withdrawn one or two hours after the injection. (Exper. I–VII.)

(2) Antialbumid produces retardation of clotting, the effect being far less marked than in the case of even smaller doses of albumoses, and furthermore requiring some time to reach its maximum effect, the results varying in proportion to the dose employed. (Exper. VIII–XV.[1])

(3) Both hemi- and antipeptone when introduced into the circulation in doses up to 0.5 gram per kilo cause no retardation or even acceleration of coagulation. Larger doses of hemipeptone[2] (1 gram per kilo) however act more like the albumoses. (Exper. XXII.)

(4) Protogelatose, as formed by gastric digestion, tends to hasten coagulation even when introduced in large doses.[3] Large doses of deuterogelatose retard coagulation quite noticeably, our results agreeing in a general way with the observations made by Arthus and Huber[4] with mixed gelatoses and gelatin-peptone.[5] True gelatin-peptone entirely free from gelatoses tends to accelerate slightly the coagulation of the blood.

Independence of arterial pressure and coagulation phenomena. — The results of the present experiments lend weight to the view that the

[1] With *antialbumid* (E) *i. e.*, a more insoluble form of the substance separating as a gelatinous coagulum when antialbumid (A) was subjected to pancreatic digestion, some peculiar results were obtained which are not included in our table. Thus, in one experiment a dog weighing 5.2 kilos received an injection of 25 c.c. of a solution of this antialbumid in weak ammonia (containing 0.29 gram of the dry antialbumid). Three blood samples taken before the injection clotted in two minutes, and arterial pressure stood at 90 mm. of mercury. One minute after the injection blood pressure fell to 10 mm. and two minutes later the animal was dead. The heart and larger blood vessels were opened and the blood was found to be clotted solid. A like result was obtained with another dog of the same body-weight, by using a somewhat larger dose of the antialbumid, namely, 0.38 gram. In a third experiment, with a dog of 13.6 kilos, using 0.33 gram of antialbumid as the total amount injected, there was a marked fall of arterial pressure, but no effect on blood coagulation. Lastly, with a fourth dog of 16 kilos having a cannula in the thoracic duct, the injection of the antialbumid (0.29 gram in 25 c.c. fluid) led to a fall of arterial pressure, a retardation of 10–12 minutes in the coagulation of the blood, a cessation of the flow of urine from the kidneys, with a small increase in the flow of lymph.

[2] Cf. the experiments on papaïn-peptone by Chittenden, Mendel, and McDermott: This journal, 1898, i, p. 271.

[3] Cf. Dastre and Floresco: Archives de physiologie, 1896, p. 402; also Floresco: *Ibid.*, 1897, p. 777.

[4] Arthus and Huber: Archives de physiologie, 1896, p. 857.

[5] With reference to the peculiar results obtained in Exper. XXXI see the section on lymph.

characteristic fall of arterial pressure and the accompanying lack of coagulability are entirely independent phenomena. The former is doubtless due to a direct and immediate action of the peptones and proteoses on the neuro-muscular apparatus of the vessels, while the latter phenomenon stands in no causal relation to the arterial fall. Thus we have observed in many experiments an entire absence of diminished coagulability, notwithstanding a pronounced fall in pressure. (Cf. experiments on peptone, gelatoses, and gelatin-peptone.) [1] Ledoux [2] has arrived at a similar conclusion, and Thompson [3] has found that a dose of Witte's "pepton" entirely insufficient to retard coagulation may still lower blood pressure, — as Fano [4] had previously found to be the case in general with acid-albumin. Finally, Dastre and Floresco [5] have quite recently demonstrated that intravenous injections of bile may entirely prevent the action of proteoses on blood coagulation without interfering with the fall of arterial pressure.

Immunity. — Since the experiments of Schmidt-Mülheim [6] and more particularly of Fano,[7] it has been known that an intravenous injection of so-called "peptone" (propeptones) which suffices to render the blood non-coagulable for a time apparently confers upon the animal a degree of immunity against subsequent injections of the "peptone." For example, if after the return of normal coagulability to the blood a second injection of albumoses is made, the latter now fails to deprive the blood of its ordinary extravascular clotting power. Furthermore, Fano demonstrated that an injection of tryptone (antipeptone from pancreatic digestion) which is without influence on the coagulation of the blood, nevertheless may render the dog immune against subsequent injections of albumose-peptone. Spiro and Ellinger [8] have likewise found that injection of antipeptone has an antagonistic influence on the action of Witte's "pepton" on blood coagulation. Grosjean [9] in his comparative study of the physiological action of propeptone and peptone reached the following conclusions: A second injection of propeptone is without

[1] See also ARTHUS and HUBER: *loc. cit.*, p. 861.
[2] LEDOUX: Archives de biologie, 1896. xiv, p. 63.
[3] THOMPSON: Journal of physiology, 1896, xx.
[4] FANO: Archiv f. Physiol., 1881, p. 277.
[5] DASTRE and FLORESCO: C. r. de la soc. de biologie, 1898, p. 458.
[6] SCHMIDT-MÜLHEIM: Archiv f. Physiol., 1880, p. 30.
[7] FANO: Archives ital. de biologie, 1882, ii, p. 146.
[8] SPIRO and ELLINGER: Zeitschr. f. physiol. Chem., 1897, xxiii, p. 137.
[9] GROSJEAN: Archives de biologie, 1892, xii.

effect on blood coagulation if it be carried out on an animal (dog) in which the effect of a previous injection has just disappeared. This is true, says Grosjean, even when the second injection is made with a large dose of the propeptone. The immunity acquired persists, for the most part, about twenty-four hours after the primary return of coagulability, when the blood again becomes coagulable; the time of coagulation not only returns to the normal but may even be shortened. With reference to purified peptone Grosjean concludes that the injection of this substance may likewise confer immunity against subsequent injections of both peptone and propeptone.

Gley and Le Bas[1] have also carried out a series of experiments on the behavior of the blood with reference to its coagulability after repeated injections of Witte's "pepton." As a result of their systematic study on a large number of dogs they conclude that in general the period of time during which the blood remains non-coagulable after a rapid injection of proteoses increases with the dose of proteoses employed per kilo of body-weight. Furthermore, the length of time during which immunity to subsequent injections persists also increases with the original dose. Thus, absolute immunity persists after return of the blood to its normal coagulability, about 15 minutes for 0.01 gram per kilo, and about 5 hours for a dose of 0.3 gram per kilo.[2] The investigations of Gley and Le Bas thus fail to confirm current statements, namely, that a preliminary injection of proteoses may confer immunity for twenty-four hours toward subsequent injections.

Arthus and Huber[3] observed that preliminary injections of gelatoses and caseoses were effective in preventing the anti-coagulating effect of subsequent large doses of the same substance. A second fall of blood pressure, however, was not prevented. Grosjean has pointed out that successive doses of both proteoses and peptones produce successively a less marked fall of arterial pressure, and a second or third injection may even be without effect on this function; a condition attributed to a degree of immunity acquired by the preliminary injection. It is to be noted, however, that in Gros-

[1] Gley and Le Bas: Archives de physiologie, 1897, p. 848. The experiments of Contejean (*Ibid.* 1895) are not referred to above since they have only an indirect bearing on this subject.

[2] Gley and Le Bas: *loc. cit.*, p. 860.

[3] Arthus and Huber: Archives de physiologie, 1896, p. 857.

TABLE OF RESULTS BEARING UPON SO-CALLED "PEPTONE" IMMUNITY.

Experiment.	Weight of animal: kilos.	First Injection.		Time between first and second injection.	Second Injection.		Coagulation of the Blood.				Arterial Pressure.		
		Grams per kilo injected.	Nature of the substance employed.		Grams per kilo injected.	Nature of the substance employed.	Prior to second injection.	5 minutes after	15 minutes after.	30 minutes after.	Prior to second injection.	5 minutes after	15 minutes after.
I	8.8	0.3	Antialbumoses (B)	2¾h	0.3	Witte's peptone	10	20	30		110	60	100
VIII	7.0	0.3	Antialbumid (A)	50	0.3	" "	45				90	22	
X	5.4	0.3	" "	2h	0.3	Antialbumid (A)	9	1h			130	120	150
X			" "	20	0.3	Witte's, 3d injection		30			150	75	135
XV	6.8	0.07	Antialbumid (F)	45	0.3	Witte's peptone	5	10			100	25	40
XVI	9.0	0.3	Antipeptone (D)	1½h	0.25	" "	3	1½h	—	27	160	50	120
XX	6.5	0.3	Hemipeptone (I)	1h	0.1	Heteroalbumose (H)	7	5	4	8	130	55	75
XXI	13.5	0.5	" "	2¼h	0.2	" "	2	3	2		145	85	110
XXIV	5.0	1.5	Protogelatose (J)	6h	0.1	" "	4		—		130	55	100
XXVI	6.0	0.6	Gelatin-peptone (L)	1½h	0.3	Witte's peptone	2	4	—		115	30	60
XXIX	15.0	0.7	Deuterogelatose (K)	2½h	0.1	Heteroalbumose (H)	2	6	6		150	60	80

NOTE. — Figures in the table referring to time denote minutes, except when followed by h, when they denote hours. Under arterial pressure, the figures denote millimetres. Under coagulation of blood, samples marked — were still fluid twenty-four hours after the experiment. The numbers of the experiments correspond with those given in the preceding tables.

jean's experiments the injections were undertaken at relatively brief intervals.

In the preceding table are presented protocols from a few of our own experiments bearing on the problem of peptone-immunity. The data presented are somewhat incomplete and fragmentary, still a few facts are clearly demonstrated. Thus it is seen that the injection of active proteoses, such as pure heteroalbumose or the mixed proteoses present in Witte's "pepton," within an hour or two after injection of the substances enumerated in the table fails to develop the characteristic clot-preventing power of the blood. There is to be sure some slight retardation of coagulation, thus indicating that the so-called immunity is by no means complete; but the effect produced by the later injections is very slight indeed as compared with the ordinary action of these products. Experiments XX, XXII, XXIX, XV, and I afford evidence of the truth of this assertion. In other cases, as in Experiments XVI and XXVI, the immunizing effect is manifested by a delay in the appearance of retarded coagulation. Thus in the two experiments just referred to, a considerable period elapsed before the blood withdrawn from the vessels assumed the characteristics of "peptone-blood." It would almost seem as if the results indicated a deficiency of the clot-preventing substances in the body after the preliminary injection, and that the latter are only *slowly* produced under the influence of the second injection.

With reference to the effects of a second injection of proteoses on arterial pressure, our results clearly show that mean arterial pressure is lowered somewhat by the second injection, thus indicating that immunity in this direction is by no means complete. That the first injection, however, does give rise to some degree of immunity is clearly shown by the fact that the second fall of pressure is noticeably less than the first one, and by the fact that the return of pressure to the normal follows somewhat more rapidly than after the first injection. Our results thus confirm, in part, Grosjean's observations, and extend them by demonstrating that the immunity acquired is by no means characteristic of specific products *injected*, but rather due to a specific reaction produced in each case within the organism itself. Delezenne[1] has arrived at a similar conclusion from quite different observations. Lastly, Dastre and Floresco[2] have recently shown that the power of conferring immunity to "peptone" injections is common

[1] Delezenne: Archives de physiologie, 1897, pp. 657-659.

[2] Dastre and Floresco: C. r. de la soc. de biologie, 1898, p. 457.

to a large number of substances, *i. e.*, urine, bile, salts of iron, calcium chloride, etc.

Influence on lymph-flow, etc. — Since Heidenhain's[1] masterly researches on the action of lymphagogues there has been, so far as we recall, no attempt to compare the lymphagogic action of the individual proteoses and peptones. It is well known that various substances which prevent clotting of the blood simultaneously increase the flow of lymph from the thoracic duct; the substances studied, however, have been for the most part merely extracts of various organs and tissues (leech-extract, extracts of crayfish and mussels). A systematic study of the active constituents of these extracts would be of great interest.

The following table contains the results of several of our experiments on the action of certain specific proteid and albuminoid derivatives on the flow and composition of the lymph. All the animals experimented on were dogs, and were deprived of food for 24–36 hours prior to the experiment.

The results of our experiments justify the following conclusions:

(1) Antialbumid, hemipeptone, heteroalbumose, and deuterogelatose may act as true lymphagogues. This is demonstrated not only by the marked increase produced in the flow of lymph from the thoracic duct, but likewise by the characteristic changes resulting in the composition of the fluid, namely, increase in total solids, the ash-content remaining constant.[2] In every case where an increased flow of lymph occurred, the fluid grew slightly reddish in color and its clotting was markedly delayed, — two phenomena characteristic of "lymphagogic" lymph.

(2) In every case where the lymph was thus diverted from the general circulation the clotting time of the blood was only slightly, if at all, affected by the injection, while the coagulability of the lymph was delayed. This agrees with the observations of Gley and Pachon,[3] who announced that the ligature of the lymphatics of the liver prevents the ordinary clot-retarding action of albumose injections in dogs; and with the observations of Spiro and Ellinger, similar to

[1] Heidenhain: Archiv f. d. ges. Physiol, 1891, xlix, p. 216.

[2] Cf. Heidenhain: *loc. cit.*

[3] Gley and Pachon: C. r. de l'acad. des sciences, 1895, p. 383; Archives de physiologie, 1895, p. 711; C. r. de la soc. de biologie, 1895, p. 741; *Ibid.*, 1897. Cf. also Starling: Journal of physiology, 1895, xix, p. 15; Delezenne: Archives de physiologie, 1896, p. 657.

TABLE SHOWING INFLUENCE ON LYMPH-FLOW, ETC.[1]

EXPERIMENT.	TIME.	Lymph-flow in 10 min.; c.c.	Total solids of lymph; per cent.	Ash of lymph; per cent.	Clotting time of lymph; min.	Clotting time of blood; min.	Arterial pressure in mm. Hg	Urine flow in 30 min.; c.c.
Antialbumid (A) [Exper. XII.] Dog of 14 kilos. Injection of 0.15 gram per kilo body-weight.	Immediately before injection . . .	1.2	4.90	0.85	10	4	140	
	Immediately after injection . . .	10.0	6.73	0.85	?	57	90	
	One half hour after injection . . .	3.0	6.10	—	?	17	130	
Hemipeptone (I) [Exper. XXI.] Dog of 13.5 kilos. Injection of 0.5 gram per kilo body-weight	Immediately before injection . . .	4.5	4.47	0.87	10	3	130	4.0
	Immediately after injection . . .	17.0	5.60	0.86	180	3	40	15.0
	One half hour after injection . . .	5.0	4.81	—	1	4	130	
HETEROALBUMOSE (H)[2] 0.2 gram per kilo injected; 2½ hours after the injection of hemipeptone.	Immediately after second injection	10.5	—	—	—	3	85	none
Deuterogelatose (K) [Exper. XXVII.] Dog of 21 kilos. Injection of 0.35 gram per kilo body-weight.	Immediately before injection . . .	4.0	5.77	0.85	30	2	115	1.0
	Immediately after injection . . .	8.6	5.69	0.89	15	2	115	10.0
	One half hour after injection . . .	4.7	4.77	—	15	½	120	
Deuterogelatose (K) [Exper. XXIX.] Dog of 15 kilos. Injection of 0.7 gram per kilo body-weight.	Immediately before injection . . .	3.0	6.58	0.92	10	3	130	5.0
	Immediately after injection . . .	15.0	7.78	0.93	90	4	60	15.0
	One half hour after injection . . .	7.2	6.24	—	20	2	150	7.0
HETEROALBUMOSE (H)[2] 0.1 gram per kilo injected; 2½ hours after the injection of deuterogelatose.	Immediately after second injection .	15.0	7.35	—	50	6	60	none
Heteroalbumose (H) [Exper. VII.] A dog of 9.8 kilos received an injection of 0.2 gram per kilo body-weight. This was followed by a pronounced fall of arterial pressure; the blood samples taken one hour after the injection failed to clot in twenty-four hours. Owing to an accident to the cannula the lymph-flow could not be measured. There was, however, a pronounced increase after the injection.								

[1] The experiments are numbered to correspond with the numbers used in the other tables.

[2] Second injection.

our own, on dogs with the thoracic duct cannulized.[1] In other words, the observations lend favor to the view that the anti-clotting substance is formed in the liver and reaches the blood mainly through the lymph.

(3) A second injection gives rise to a marked renewal of lymph-flow, independent of any action on blood coagulation. The two Experiments XXI and XXIX do not, however, permit the immediate conclusion that the lymphagogic action and clot-preventing phenomena are entirely independent,[2] inasmuch as the diversion of the lymph from the circulation may prevent the anti-coagulating substance from reaching the blood.[3] Since some dogs are naturally immune to albumose injections, no far reaching conclusions can be drawn from the data at hand.

(4) Ordinary doses of deuterogelatose (0.3 gram per kilo) give rise to no marked lymphagogic action, large amounts being required to produce characteristic effects. This corresponds with the other observations made on the physiological action of this substance. With heteroalbumose, lymphagogic action is as strongly marked as the action on blood coagulation and arterial pressure already noted.

(5) Previous injection of hemipeptone or deuterogelatose is entirely without influence on the lymphagogic action of heteroalbumose; the latter producing a marked increase in the flow of lymph when injected a short time after the action of the primary substances has ceased.

Influence on the flow of urine, etc. — In conformity with previous observations our results, shown in the following table, lead to the conclusion that the albumoses, owing to their marked influence in lowering blood pressure, tend to cause a complete cessation of the flow of urine. With heteroalbumose, however, the results seemingly justify a somewhat different conclusion. Thus, in three experiments, (XXI, XXIV, and XXIX) some hours after the animals had received injections of substances which act as diuretics and lympha-

[1] SPIRO and ELLINGER: Zeitschr. f. physiol. Chem., 1897, xxiii, p. 135.

[2] Cf. STARLING: Journal of physiology, 1893, xiv, p. 140; also GLEY and PACHON: Archives de physiologie, 1897, p. 861.

[3] ASHER and BARBÈRA (Zeitschr. f. Biologie, 1898, xxxvi, p. 154) have put forward the opinion that lymphagogues are substances which stimulate the activity of various organs and especially glands. In agreement with this view they find that "peptone" is an active "cholagogue." Starling and others, however, have questioned this conclusion. See Proceedings of Fourth International Physiological Congress. Centralbl. f. Physiol., 1898, xii.

gogues, an injection of heteroalbumose was given. This resulted in a complete stoppage of the flow of urine; not a drop of urine was obtained within the half hour or more after this injection, *although the fall of arterial pressure was comparatively slight and transitory.* Hence, it follows that heteroalbumose, unlike the other albumoses, must exert some influence upon the action of the kidneys independent of blood pressure; possibly, a specific influence upon the secretory activity of the epithelial cells. In any event heteroalbumose may check the formation of urine even when arterial pressure is not materially affected. On the other hand, as already pointed out, heteroalbumose stimulates in marked degree the flow of lymph. (See preceding table on lymph-flow.)

Antialbumid acts much like ordinary albumoses in checking the secretion of urine, but if comparison is made, by reference to the table showing arterial pressure, of the influence on renal secretion and mean arterial pressure, as in Experiments VIII, X, and XI, it will be seen that the inhibitory effect on the secretory action of the kidneys is considerably greater than would naturally be expected from the short period of diminished arterial pressure. This fact obviously suggests a specific action on the part of antialbumid upon the kidneys independent of pressure.

On the other hand, large doses of hemipeptone, protogelatose, and deuterogelatose have a marked diuretic effect, increasing not only the volume of urine secreted in a given time, but likewise the content of solid matter, as shown by the very decided increase in specific gravity.[1] Coincident with the diuresis it will be observed that mean arterial pressure is slightly raised under the influence of the substance. The marked rise in the specific gravity of the urine, noticed after the injections, is associated mainly with the rapid excretion of the substance thrown into the circulation. As has been previously noted[2] in this connection, a primary proteose introduced into the blood is eliminated, in part at least, as a secondary proteose. In other words, the primary body undergoes hydration in the kidney[3] or elsewhere, and is excreted through the urine in slightly altered form. Thus, in Experiment XXIV with protogelatose not only was the rate of flow

[1] See CHITTENDEN: Science N. S. v, June 11, 1897, p. 902; also THOMPSON: Journal of physiology (Proceed. Physiol. Soc.), 1898, xxii, p. xi.

[2] NEUMEISTER: Zeitschr. f. Biologie, 1887, xxiv, p. 272; also CHITTENDEN, MENDEL, and MCDERMOTT: This journal, 1898, i, p. 266.

[3] NEUMEISTER: *loc. cit.*

TABLE SHOWING EFFECTS UPON URINARY SECRETION.

Experiment.	Weight of animal; kilos.	Grams per kilo injected.	Nature of the substance injected.	Urine in 30 mins. before injection.		Urine in 30 mins. after injection.		Remarks.
				Vol.	Sp. gr.	Vol.	Sp. gr.	
				c.c.		c.c.		
VIII	7.0	0.3	Antialbumid (A) . . .	19.2		2.6		
X	5.4	0.3	" " . . .	7.2		0		
XI	7.6	0.14	" " . . .	7.0		2.9		
XVI	9.0	0.3	Antipeptone (D) . . .			30.0		Strong biuret reaction.
XVII	6.5	0.3	" " . . .	6.7		10.5		" " "
XVIII	9.0	0.3	" " . . .	4.2	1026	43.5	1016	" " "
XIX	11.2	0.3	Hemipeptone (I) . . .	46.2	1031	38.0	1036	Urine almost black.
XX	6.5	0.3	" " . . .	4.0		4.0		No biuret; normal color.
XXI	13.5	0.5	" " . . .	4.0	1067	15.0	1042	No biuret; dark color.
XXII	6.4	1.0	" " . . .	17.5	1035	55.0	1035	" " "
XXIII	5.0	0.3	Albumose-like body (M)	2.5		0		" " "
XXIV	5.0	1.5	Protogelatose (J) . . .	13.8	1021	32.0	1061	Dark color. Contained deuterogelatose and peptone.
XXV	4.2	0.3	Gelatin-peptone (L) . .			25.0		No biuret; dark color.
XXVI	6.0	0.6	" " " . .	15.0	1046	10.0	1045	" "
XXVII	21.0	0.35	Deuterogelatose (K) . .	1.0		10.0		
XXVIII	7.0	0.5	" " . .	8.0	1046	10.0	1093	Dark urine. Contained deuterogelatose and peptone.
XXIX	15.0	0.7	" " . .	5.0	1062	15.0	1093	
XXX	6.5	1.0	" " . .	2.1		4.5		

of the urine more than doubled, but the specific gravity rose from 1021 to 1061. Examination of the urine showed the presence of a large amount of gelatose, but this was mainly deuterogelatose. Further, on saturating the urine with ammonium sulphate under appropriate conditions for removal of all the gelatoses, and testing the filtrate by the biuret test, a distinct reaction for true gelatin-peptone was obtained. Five and a half hours after the injection the urine still contained an appreciable amount of gelatose. After injection of deuterogelatose the urine was likewise found rich in gelatin-peptone, while some unaltered gelatose was also found. This excretion of gelatoses through the urine as gelatoses and gelatin-peptone was quite marked, and we were able to separate from such urines several grams of fairly pure gelatose, thus showing that the elimination through this channel was not inconsiderable. If it is true, as suggested by Neumeister, that the hydration of a primary proteose to secondary proteose and the hydration of the latter to true peptone in these cases is due to the presence of pepsin in the kidneys acting in conjunction with acid temporarily present, then our results testify to the extreme vigor of such action, and indirectly suggest the presence of considerable active enzyme. Whether this hydrolytic action is truly due to the presence of an enzyme our results afford no answer. In this connection it will be remembered that Hofmeister[1] observed that when amphopeptone was injected subcutaneously four fifths of the substance appeared in the urine unaltered. Neumeister[2] likewise noted that amphopeptone was excreted as such through the urine. In a paper from this laboratory,[3] however, attention was called to the fact that peptone formed through the action of papain was much less markedly eliminated through the urine than deuteroalbumose; this statement being based upon the much weaker biuret reaction given by the urine. In conformity with this observation we have noted in the present experiments with hemipeptone and gelatin-peptone that after the intravenous injection of these substances their presence in the urine could not be detected by the biuret reaction. Whether this fact is to be taken as an indication of their excretion by the urine in the form of amido-acids, etc., formed through proteolytic action in the kidneys, or whether it is due to decomposition taking place elsewhere or to a storing up within the body we cannot say.

[1] HOFMEISTER: Zeitschr. f. physiol. Chem., 1881, v, p. 131.

[2] NEUMEISTER: Zeitschr. f. Biologie, 1887, xxiv, p. 287.

[3] CHITTENDEN, MENDEL, and MCDERMOTT: This journal, 1898, i, p. 275.

OBSERVATIONS ON THE CHEMICAL NATURE AND GENERAL PROPERTIES OF THE PROTEID CLEAVAGE PRODUCTS USED IN THE PRECEDING EXPERIMENTS.

Among the many earlier results obtained in the attempt to throw light upon the chemical nature of the proteid molecule those recorded by Schützenberger[1] were particularly suggestive, especially from a physiological standpoint. The breaking down of the proteid molecule into two equal parts by the action of 3 to 5 per cent sulphuric acid at 100° C. naturally suggested the possible existence of two distinct groups in the mother proteid; a suggestion which was intensified by the observations of Kühne[2] that these two cleavage or alteration products behaved quite differently toward alkaline pancreatic juice. Schutzenberger through his hydrolysis of coagulated egg-albumin by dilute sulphuric acid obtained an insoluble residue equal to about 50 per cent of the original proteid, to which he gave the name of hemiprotein, while the 50 per cent of soluble matter was represented by a row of substances among which leucin, tyrosin, peptone, and albumoses (as now termed) were conspicuous. As Schützenberger pointed out, it was quite evident that these latter products resulting from this hydrolysis represented a half of the proteid molecule much less stable than that portion of the proteid from which the so-called hemiprotein was derived. This more readily decomposable half of the proteid he considered as represented by a body which he termed hemialbumin, and which by continued hydrolysis gave rise to peptone and amido-acids. These conclusions were verified in part, and extended by Kühne in his study of the action of the proteolytic enzymes upon these two bodies; *i. e.*, hemiprotein and hemialbumin. The former was found to be slowly digestible by pepsin-hydrochloric acid, while by alkaline pancreatic juice it was eventually transformed in great part into a peptone without any trace of amido-acids being formed.[3] In other words, the substance was remarkably resistant to that secondary action of the pancreatic enzyme by which pancreatic proteolysis is especially characterized. On this account Kühne suggested the name of antialbumid as more expressive of the general

[1] SCHÜTZENBERGER: Bulletin de la Société chimique de Paris, 1875, xxiii, and xxiv.

[2] KÜHNE: Verhandl. d. Naturhist.-Med. Vereins zu Heidelberg, 1877, i, p. 236; KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1883, xix, p 159.

[3] KÜHNE and CHITTENDEN: *loc. cit.*

nature of the substance than the term hemiprotein, and by this name the substance has generally been known for the past twenty years. The so-called hemialbumin of Schutzenberger was found by Kuhne to be readily digestible in artificial gastric juice, giving rise to peptone, while by the action of pancreatic juice leucin and tyrosin were formed in abundance. To the hemialbumin of Schutzenberger, Kuhne gave the name of hemialbumose, which prevailed for a time until it was found that this substance was in reality a mixture of several related substances, to which the name of albumoses was applied.[1] Through these researches, followed by many others that need not be enumerated here, the view that in the native proteid molecule there are present two distinct atomic complexes has gradually gained credence, and as a result the prefixes hemi and anti have been, and are, widely used to designate inner differences in the various hydrolytic cleavage products of the proteids, whether formed by the action of boiling dilute acids or by the action of proteolytic enzymes. A hemi body has thus come to mean a substance which under the influence of trypsin will break down into crystalline amido-acids and other simple products, while an anti body fails to yield any such simple products under the action of trypsin. The researches of Neumeister[2] and others have shown, however, that when this crucial test is applied to the specific proteoses formed in gastric digestion, for example, there is invariably an admixture of so-called hemi and anti groups; not an equal mixture, — for protoproteose is apparently composed in great part of hemi groups with only a small admixture of anti groups, while heteroproteose is made up chiefly of anti groups, as judged by the behavior of the respective bodies toward trypsin. In the amphopeptone of gastric digestion, on the other hand, we have apparently a commingling in equal proportion of hemi and anti groups, for if this substance is digested for some time with a vigorous pancreatic juice, approximately one half breaks down into crystalline decomposition products, leaving a residue of peptone resistent to the further action of trypsin, *i. e.*, an antipeptone. How far these terms hemi and anti are to be considered as representing real differences in chemical constitution is very uncertain. It may be that the differences are more apparent than real, and that the characteristic behavior toward trypsin is the result of more or less superficial peculiarities. Granting that this is so, the terms still have significance as

[1] KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1884, XX. p. 11.

[2] NEUMEISTER: Zeitschr. f. Biologie. 1887. XXIII. p. 380.

indicating physiological differences of some moment and are thus of value. Certainly the view at one time suggested that an anti body contained no aromatic groups capable of yielding tyrosin under any conditions is not tenable. To be sure, a pure antipeptone gives little or no Millon's reaction, but antialbumid boiled for some time with 10 per cent sulphuric acid will yield considerable tyrosin, although when acted upon by an alkaline solution of trypsin no tyrosin whatever results. Thus, Dr. Alice H. Albro, working in this laboratory with antialbumid made from coagulated egg-albumin, found on boiling the substance with 10 per cent sulphuric acid for 80 hours that 2.7 per cent of tyrosin was formed together with considerable leucin. In this connection it will be remembered that Erlenmeyer and Schöffer obtained 1 per cent of tyrosin by boiling egg-albumin with sulphuric acid, while Schützenberger obtained 2.0 per cent by heating egg-albumin at 160–200° C. with baryta water for 4 to 6 days.[1] Obviously, one cannot draw very sharp comparisons between these several results, but it is quite clear that antialbumid made from coagulated egg-albumin by the action of 4 per cent sulphuric acid at 100° C. yields fully as much tyrosin on decomposition with a strong mineral acid as the mother proteid. Hence the difference in the result when these two substances are treated with active pancreatic juice must be due, not to the lack of the characteristic aromatic group in the antialbumid molecule, but rather to some peculiar state of combination of the atoms or radicles which prevents the formation of tyrosin or other amido-acid through the action of trypsin. In the case of antialbumid formed by the hydrolytic action of a dilute acid it might perhaps be claimed that it is purely an artificial product, and that its resistance to the secondary action of trypsin is merely the result of some slight alteration of the original proteid attendant upon the process of cleavage. As opposed to this idea, however, we have the well-known fact that the original coagulated albumin placed directly in alkaline pancreatic juice breaks down step by step to leucin, tyrosin, etc., leaving at the end a large amount — perhaps 50 per cent — of a peptone (antipeptone) which resembles the peptone resulting from the pancreatic digestion of antialbumid. In other words, there is abundant reason for believing that the so-called anti group is contained as such in the native proteid, and that in the cleavage with dilute sulphuric acid it is split off as antialbumid. while in the pancreatic digestion of the native proteid it is isolated as antipeptone through

[1] See Hermann's Handbuch der Physiologie, v (2), p. 212.

conversion of the so-called hemi groups into amido-acids and nitrogenous bases.

While it is thus possible to isolate with some degree of purity bodies belonging to or derived from the anti half of the proteid molecule, less satisfactory is the isolation of the so-called hemi bodies. From the amphopeptone formed in gastric digestion, the antipeptone can be separated through destruction of the hemi groups, but there is no way known of isolating the hemipeptone from this mixture. A method promising better results is the hydrolytic cleavage of the native proteid with dilute acid, accompanied as it is by the splitting off of the anti groups as antialbumid. While this results in the formation of soluble hemialbumoses and hemipeptone, which can easily be separated, it unfortunately happens that the antialbumid formed also undergoes hydration to some slight degree with consequent formation of some soluble antialbumoses and antipeptone. These latter bodies once mixed with the corresponding hemi products cannot be separated, and hence are an ever-present impurity. Still, this method affords the only way, at present, of obtaining the so-called hemialbumoses and peptone, and we have had recourse to it as the only available method, understanding, however, that the several products are by no means wholly free from corresponding anti bodies.

As already stated in the first part of this paper, it seemed very desirable in view of the importance attaching to the physiological action of the various digestive products of the proteids to ascertain as definitely as possible the part played by the several components of the ordinary products of proteolytic action. With this end in view we have devoted particular attention to the anti products, and in order to insure their freedom, so far as possible, from the so-called hemi groups we have made use of antialbumid as the mother substance in the preparation of antialbumoses and antipeptone.

Hydrolysis and cleavage of coagulated egg-albumin with formation of antialbumid, etc. — In obtaining sufficient material for our purpose it was necessary to repeat the process several times, but the general line of procedure in each case was as follows: The whites of twelve dozen eggs were coagulated by pouring them slowly into a large volume of boiling water acidified slightly with acetic acid, the coagulum collected on a cloth filter and washed with large quantities of boiling water. It was then pressed as dry as possible and placed in a large flask with 4 per cent sulphuric acid (360 grams of the moist coagulum in 1200 c.c. of the acid). This mixture was then heated at

100° C. in a large steam sterilizer (Arnold's), with occasional agitation, for 12 to 15 hours, after which the gelatinous antialbumid was separated from the acid solution (containing the hemi bodies) by filtration through paper and freed from all soluble matter by long continued washing with water. The washing was continued until the wash-water reacted only faintly acid. In some cases the antialbumid was again heated at 100° C. for 12 hours with a fresh lot of 4 per cent sulphuric acid. The crude antialbumid was next warmed at 40° C. for forty hours with a large volume of an active solution of pepsin-hydrochloric acid for the complete removal of adherent hemi bodies or of any unaltered egg-albumin. The residual antialbumid was then filtered off and washed thoroughly with distilled water until the washings were free from chlorine. The product was next dissolved in a solution of 0.5 per cent sodium carbonate, the fluid filtered through paper, and the antialbumid reprecipitated by neutralization with 0.2 per cent hydrochloric acid, after which it was again washed with water until free from chlorine, and lastly with 95 per cent alcohol. This product constitutes *antialbumid* (*A*).

In separating the so-called hemialbumoses from the 4 per cent sulphuric acid solution, the clear filtered fluid was neutralized with ammonia and evaporated to a convenient volume, the neutralization precipitate filtered off, and the further concentrated fluid eventually saturated boiling hot with ammonium sulphate in acid, neutral and alkaline reaction according to the method suggested by Kühne.[1] The precipitate of mixed albumoses was dissolved in water, a little thymol added, and the fluid dialyzed in running water until the sulphate was entirely removed. When the dialysis was completed a small quantity of heteroalbumose was found adherent to the parchment. This was therefore filtered off, washed thoroughly with water, lastly with alcohol, and dried. This product constitutes *heteroalbumose* (*H*). Assuming the correctness of Neumeister's statement that heteroalbumose as formed in pepsin-proteolysis is composed mainly of anti groups, it suggests that the formation of this substance in the hydrolysis with sulphuric acid may be taken as a measure of the extent to which in this hydrolysis anti groups are split off in forms other than antialbumid. On the other hand there is perhaps no logical reason why a heteroalbumose might not be formed under this peculiar method of hydrolysis, out of hemi groups mainly.

The above fluid freed by dialysis from ammonium sulphate and

[1] KÜHNE: Zeitschr. f. Biologie, 1892, xxix, p. 1.

heteroalbumose, after filtration from the latter substance, was concentrated to a syrup, precipitated with alcohol, and the precipitate washed thoroughly with hot alcohol and dried. This product constitutes *hemialbumoses* (*G*), and is composed of a mixture of hemi protoalbumose and hemi deuteroalbumose.

The hemipeptone contained in the ammonium sulphate-saturated fluid after separation of the mixed albumoses was obtained as follows: the fluid was concentrated somewhat, cooled, and the crystals of ammonium sulphate separated, this operation being repeated four times, thus accomplishing the removal of a large amount of the contained sulphate. During the repeated heating of this solution a small quantity of a dark, oily, albumose-like substance was obtained, which was filtered off and freed from ammonium sulphate, by the method used in the purification of the peptone, *i. e.*, with barium carbonate, etc. The product so isolated constitutes the *albumose-like body* (*H*). The solution was next treated with barium hydroxide until the larger portion of the sulphate was precipitated, after which the last portions of the ammonium salt were removed by warming the solution with pure barium carbonate. The filtered fluid was then treated with very dilute sulphuric acid, drop by drop, until the last trace of dissolved barium was removed and the filtrate concentrated to a small volume, precipitated with alcohol and the precipitate washed repeatedly with boiling alcohol. This product constitutes *hemipeptone* (*I*).

Formation of antialbumoses by the action of pepsin-acid on pure antialbumid. — When antialbumid is warmed at 40° C. with an active solution of pepsin-hydrochloric acid (0.2 per cent HCl) there results a slow but gradual digestion of the proteid with formation of soluble antialbumoses together with a trace of peptone. Using antialbumid (A) as the mother substance, digestion with pepsin-acid was carried on for some days, the undigested residue filtered off, the solution neutralized with sodium carbonate, concentrated, and the albumoses separated by saturation of the boiling solution with ammonium sulphate. The gummy precipitate so obtained was dissolved in water, dialyzed in running water until free from sulphate, then concentrated to a syrup and precipitated with alcohol. The product was a mixture of protoalbumose and deuteroalbumose, and constitutes the preparation called *antialbumoses* (*B*).

Antialbumoses and antipeptone formed by the action of an alkaline solution of trypsin on antialbumid. — Antialbumid (A) was dissolved in 0.5 per cent sodium carbonate and the solution warmed at 40° C.

for five days with a purified solution of trypsin,[1] also in 0.5 per cent sodium carbonate, thymol being added to prevent putrefaction. After the clear solution had been at 40° C. for a few hours it became opaque and soon formed a thick jelly due to the separation of a portion of the antialbumid in a slightly modified form, as has frequently been described in other places.[2] At the expiration of the five days a large proportion of this insoluble matter had disappeared, but there still remained an appreciable amount of the substance, which was filtered off, thoroughly washed with water, and dried. This constitutes *antialbumid* (*E*). The alkaline solution containing the albumoses and peptone was neutralized with dilute hydrochloric acid, the slight precipitate removed by filtration, and the fluid concentrated to a convenient volume. The antialbumoses and antipeptone were then separated by ammonium sulphate and isolated exactly as described under the head of hemialbumoses and hemipeptone. The antipeptone, however, owing to the presence of sodium chloride, was further purified by dialysis until the chloride was entirely removed. The product is *antipeptone* (*D*).

The antialbumoses, indicated as *antialbumoses* (*C*), were a mixture of proto and deuteroalbumose. This is a matter of some moment chemically, for it is generally assumed that in trypsin-proteolysis primary proteoses are not formed; that the proteid passes directly to deuteroproteose and thence into true peptone, etc.[3] Further, it is implied in the scheme of pepsin-proteolysis generally accepted by writers on this subject, that antialbumid undergoing gastric digestion is transformed directly into antideuteroalbumose without any intermediate formation of a primary proteose.[4] This, however, is not the case; certainly not where pure antialbumid is undergoing digestion. Thus, we have observed that in the digestion of antialbumid with both gastric juice and pancreatic juice, the albumoses formed and separated contained a large amount of protoalbumose; the neutral

[1] The trypsin solution was prepared by warming 40 grams of Kühne's dry ox pancreas in 400 c.c. 0.1 per cent salicylic acid solution for 24 hours, making the extract alkaline (0.25 per cent Na_2CO_3) and warming this at 40° C. for 48 hours in the presence of thymol. The extract was next dialyzed for some days from both alkaline and acid (acetic acid) solution, then evaporated to dryness at 40° C. and the residue taken up with a very little cold water. This solution, filtered, contains fairly pure trypsin.

[2] KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1883, xix. p. 166.

[3] See NEUMEISTER'S Lehrbuch d. physiol. Chem., 1897, p. 247.

[4] See NEUMEISTER: Zeitschr. f. Biologie, 1887, xxiii. p. 391.

solution of the albumoses yielding a very decided precipitate on saturation with sodium chloride.

The antipeptone (D) formed by the action of trypsin on pure antialbumid was found on analysis to have the following composition, when dried at 110° C.

I. 0.3003 gram substance gave 0.1812 gram H_2O = 6.70 per cent H and 0.5502 gram CO_2 = 49.96 per cent C.
II. 0.3330 gram substance gave 0.6075 gram CO_2 = 49.75 per cent C.
III. 0.2681 gram substance gave by the Kjeldahl method 0.03570 gram N = 13.32 per cent N.
IV. 0.2722 gram substance gave 0.03612 gram N = 13.27 per cent N.
V. 1.0382 grams substance gave by fusion with $NaOH$[1] and KNO_3 0.1203 gram $BaSO^4$ = 1.59 per cent S.
VI. 1.0665 grams substance gave 0.0228 gram ash = 2.14 per cent.

Percentage composition of the ash-free substance.

	I	II	III	IV	V	*Average*
C . . .	51.05	50.82	....	....	....	50.93
H . . .	6.84	....	....	....	....	6.84
N . . .	....	....	13.61	13.56	....	13.58
S . . .	....	....	....	....	1.62	1.62
O . . .	....	....	....	....	....	27.03
						100.00

This is the only analysis, so far as we are aware, of an antipeptone prepared from pure antialbumid. Some years ago Kuhne and Chittenden[2] analysed a preparation of so-called antipeptone prepared by the digestion of antialbumid with alkaline pancreatic juice, but at that time the proteoses had not been discovered and consequently the method of separation made use of did not effect the removal of the antialbumoses; hence, the product analyzed was without doubt a mixture of true antipeptone and antialbumoses. In considering the composition of this product it will be remembered that antialbumid is characterized by a comparatively high content of carbon and a low content of nitrogen (53.79 per cent C and 14.55 per cent N),[3] hence it is to be observed that in the formation of this peptone, as in all cases of peptone formation, the mother proteid loses an appreciable amount of carbon.[4] Somewhat noticeable in this product is the comparatively high content of sulphur, for peptones as a rule contain a very small

[1] Pure sodium hydroxide made from the metal sodium and free from sulphur. The fusion was made in a silver crucible over an alcohol lamp.

[2] KÜHNE and CHITTENDEN: Zeitschr. f. Biologie, 1883, xix, p. 169.

[3] KÜHNE and CHITTENDEN: *loc. cit.*, p. 167.

[4] See CHITTENDEN: Digestive proteolysis, New Haven, 1895, p. 70.

amount of this element. There was no sulphur, however, present in the mercaptan form, and the ash contained no sulphate. Neither was there any evidence of the presence of ammonium sulphate. As to the relationship in composition between this antipeptone and the antipeptone resulting from the pancreatic digestion of a native proteid it will suffice here to refer the reader to previous papers on antipeptone.[1]

Another product to be referred to under the head of anti bodies is *antialbumid* (*F*). This substance was prepared from well crystallized edestin,[2] a globulin obtained from hemp-seed, by heating the substance at 100° C. with 4 per cent sulphuric acid. The gelatinous antialbumid resulting by this process was purified by solution in 0.5 per cent sodium carbonate, etc., and then used for intravenous injection.

Hydrolysis and cleavage of pure gelatin by trypsin in neutral solution. — In this hydrolysis 200 grams of exceptionally pure gelatin prepared from the purified collagen of white fibrous connective tissue (tendons) by Dr. Van Name[3] were available. This material was dissolved in four litres of distilled water, a neutral solution of purified trypsin[4] added, together with sufficient thymol to prevent putrefaction, and the mixture warmed at 40° C. for four weeks. The object of this long-continued digestion was to insure the formation of as large an amount as possible of true gelatin-peptone, the substance usually described under this name being nothing more than a mixture of gelatoses. Further, digestion was carried on in a *neutral* fluid to obviate the necessity for removal from the resultant products of alkali salts; a process which entails great loss of substance. Careful examination of the digestive mixture at the end of the four weeks showed the complete absence of primary gelatoses. The solution was therefore concentrated and the deuterogelatose separated directly by saturation with ammonium sulphate under the conditions already referred to. The product was purified by dialysis, and eventually precipitated from a concentrated solution by strong alcohol, after which it was repeatedly boiled with alcohol for removal of any soluble extractives and then dried. This product constitutes *deuterogelatose* (*K*). The yield was 113 grams.

[1] Kühne and Chittenden: Zeitschr. f. Biologie, 1886, xxii, p. 423; Chittenden: Studies in physiological Chemistry, Yale University, 1889, iii, p. 100; Chittenden and Goodwin: Journal of physiology, 1891, xii, p. 34; Balke: Zeitschr. f. physiol. Chem., 1896, xxii, p. 248, etc.

[2] Chittenden and Mendel: Journal of physiology, 1894, xvii, p. 48.

[3] Van Name: Journal of experimental medicine, 1897, ii, p. 117.

[4] See foot-note (p. 174).

The ammonium sulphate-saturated fluid containing the true gelatin-peptone formed by trypsin was freed from the excess of ammonium salt by crystallization, the residue removed by treatment with barium hydroxide and barium carbonate as described in the preparation of hemipeptone, and the true peptone precipitated by alcohol. After thorough and repeated extraction with boiling alcohol the peptone was dried. *Gelatin-peptone* (*L*). It weighed 17 grams. The relative yield of deuterogelatose and true peptone in this long-continued hydrolysis with an active solution of trypsin constitutes striking evidence of the relatively slow production of true gelatin-peptone in trypsin-proteolysis.

The deuterogelatose in aqueous solution gave no precipitate whatever on saturation of the fluid with sodium chloride, neither did any precipitate result on addition of acetic acid to the salt-saturated fluid. Consequently, it must be considered as entirely free from protogelatose.[1] Tested with potassium hydroxide and plumbic acetate for loosely combined sulphur, both peptone and gelatose gave negative results. Negative results were likewise obtained with Millon's reagent and the xanthoproteic test. With the biuret test, strong solutions of both gelatose and peptone yielded a bright pink color. The composition of these two products is shown by the following results.

Analysis of Deuterogelatose (*K*).[2]

I. 0.2811 gram substance gave 0.1705 gram H_2O = 6.74 per cent H and 0.5201 gram CO_2 = 50.46 per cent C.

II. 0.2895 gram substance gave 0.1769 gram H_2O = 6.79 per cent H and 0.5356 gram CO_2 = 50.45 per cent C.

III. 0.3195 gram substance gave by the Kjeldahl method 0.05556 gram N = 17.39 per cent N.

IV. 0.2952 gram substance gave 0.05134 gram N = 17.39 per cent N.

V. 0.5166 gram substance gave by fusion with NaOH and KNO_3 0.0126 gram $BaSO_4$ = 0.33 per cent S.

VI. 0.3695 gram substance gave 0.0030 gram ash = 0.81 per cent.

Percentage composition of the ash-free substance.

	I	II	III	IV	V	*Average*
C . . .	50.87	50.86	....	....	....	50.87
H . . .	6.79	6.84	....	....	....	6.81
N . . .	....	....	17.53	17.53	....	17.53
S . . .	....	....	....	....	0.34	0.34
O . . .	....	....	....	....	....	24.45
						100.00

[1] See CHITTENDEN and SOLLEY. Journal of physiology. 1891, xii, p. 33.

[2] Dried at 105° C. until of constant weight.

Analysis of Gelatin-peptone (L).

I. 0.2984 gram substance gave 0.1797 gram $H_2O = 6.69$ per cent H and 0.5168 gram $CO_2 = 47.23$ per cent C.

II. 0.2134 gram substance gave 0.1286 gram $H_2O = 6.69$ per cent H and 0.3709 gram $CO_2 = 47.40$ per cent C.

III. 0.2869 gram substance gave by the Kjeldahl method 0.04827 gram N = 16.82 per cent N.

VI. 0.2168 gram substance gave 0.03661 gram N = 16.88 per cent N.

V. 0.5017 gram substance gave by fusion with NaOH and KNO_3 0.0119 gram $BaSO_4$ = 0.32 per cent S.

VI. 0.5376 gram substance gave 0.0114 gram ash = 2.12 per cent.

Percentage composition of the ash-free substance.

	I	II	III	IV	V	*Average*
C	48.25	48.42	....	....	....	48.33
H	6.83	6.83	....	....	....	6.83
N	....	....	17.18	17.24	....	17.21
S	....	....	....	....	0.33	0.33
O	....	....	....	....	....	27.30
						100.00

Comparing these results with the composition of the pure gelatin from which the gelatose and peptone were derived we have the following figures:

	Gelatin[1]	*Deuterogelatose*	*Gelatin-peptone*
C	50.11	50.87	48.33
H	6.56	6.81	6.83
N	17.81	17.53	17.21
S	0.25	0.34	0.33
O	25.24	24.45	27.30

Thus, again we have a forcible illustration of the fact that in both pepsin- and trypsin-proteolysis the formation of true peptone is associated with a marked diminution in the content of carbon.[2] The composition of the deuterogelatose, on the other hand, is seen to be essentially identical with that of the gelatin from which it was derived, thus agreeing with the observations previously recorded by Chittenden and Solley[3] on the composition of the gelatoses formed by gastric and pancreatic digestion.

The protogelatose (protogelatose *J*) tested intravenously was a specimen prepared by pepsin-proteolysis of a purified commercial gelatin,[4] and had the following composition:

C 49.98, H 6.78, N 17.86, S 0.52, O 24.86, Ash 1.98.

[1] Van Name: *loc. cit.*, p. 124.

[2] Chittenden: Digestive proteolysis, New Haven, 1895, p. 71.

[3] Chittenden and Solley: Journal of physiology, 1891, xii, p. 23.

[4] Chittenden and Solley: *loc. cit.*, p. 28.

Addendum.

Since this work was completed two exceedingly interesting and important papers on antipeptone have appeared from Kossel's laboratory,[1] which have a direct bearing upon the purity of antipeptone formed from blood-fibrin by digestion with an alkaline pancreatic extract. Kutscher's results tend to show that ordinary antipeptone formed from blood-fibrin by pancreatic digestion and purified by the methods ordinarily made use of contains a large proportion of impurities composed in great part of the nitrogenous bases arginin, histidin, and a new body as yet undescribed, together with aspartic acid and some leucin and tyrosin. Assuming this to be true of all antipeptones formed from native proteids by direct digestion with pancreatic juice, it follows that the products of this kind hitherto tested physiologically are open to the suspicion of being unsuitable for experiments of this nature. How far the same criticism will apply to the antipeptone employed by us in the present experiments is uncertain. It would seem, however, that the antipeptone prepared from antialbumid might perhaps be free from this objection or at least contain a far smaller amount of impurity, since in the hydrolysis and cleavage of egg-albumin in the preparation of antialbumid the more easily decomposable half of the proteid molecule would be at once eliminated. Consequently in forming antipeptone from pure antialbumid by trypsin-proteolysis it is quite possible that the resultant peptone is free from the objectionable features of ordinary antipeptone owing to the nature of the mother substance. If such is the case our present experiments possess a double value. In partial justification of the belief that antipeptone prepared from antialbumid is less liable to contain the impurities referred to is the fact that the well known bodies leucin and tyrosin, so common among the products formed in the breaking down of a native proteid with trypsin and usually associated with the nitrogenous bases, are never found in the proteolysis of pure antialbumid. Further, in the original analyses of antipeptone made by Kühne and Chittenden,[2] it was found that purification by phosphotungstic acid tended to raise decidedly the content of nitrogen; a fact which Kutscher explains by assuming the more complete precipitation of the nitrogenous bases by this reagent. Thus, in a preparation

[1] Kutscher: Zeitschr. f. physiol. Chem., 1898, xxv, p. 195; Kutscher: *ibid*, 1898, xxvi, p. 110.

[2] Kühne and Chittenden: Zeitschr. f. Biologie, 1886, xxii, p. 435.

of fibrin-antipeptone containing 16.58 per cent of nitrogen, precipitation by phospho-tungstic acid resulted in raising the content of nitrogen in the substance to 18.28 per cent.[1] In other words, this method of purification led, according to Kutscher, to an increase in the proportion of impurities. This being so, the higher content of nitrogen in an antipeptone is to be looked upon with suspicion. Accepting the truth of this statement, the extremely low content of nitrogen in the antipeptone from antialbumid (13.58 per cent) may perhaps be taken as additional evidence of the freedom of the peptone from the objectionable nitrogenous bases. It is not to be understood from this hypothesis that the anti bodies, antialbumid or antipeptone, will not yield nitrogenous bases on decomposition. The real distinction between a hemi and anti body is based upon the behavior of the substance toward trypsin. As we have already pointed out, antialbumid which will not yield tyrosin on treatment with trypsin, will yield considerable tyrosin on decomposition with a strong mineral acid at 100° C. So likewise, these anti bodies may yield a large proportion of the nitrogenous bases on decomposition with a boiling mineral acid while wholly resistant to the action of trypsin.

In this connection it has seemed to us important to ascertain how large a yield of nitrogenous bases can be obtained from antialbumid by decomposition with a mineral acid, and also to compare the results with those obtainable by a like decomposition of a hemi body. For this purpose, 10 grams each of antialbumid, hemialbumoses, and hemipeptone were boiled for 96 hours with 40 c.c. of 20 per cent hydrochloric acid with addition of a little stannous chloride. The solutions were then diluted with water, the tin removed by hydrogen sulphide, and the filtrates evaporated to syrupy consistency. The residues were then dissolved in water, and each made up to 250 c.c. Measured portions of these solutions were used for the determination of total nitrogen, nitrogen as ammonia, and basic nitrogen precipitated by phospho-tungstic acid, following the methods used by E. Schulze.[2] Following are the results obtained:

Antialbumid (A)

Total nitrogen in the fluid	1.2745 grams.
Nitrogen in phospho-tungstic acid precipitate	0.2782 "
Nitrogen in the form of ammonia	0.0585 "
Basic nitrogen, organic	0.2197 "

17.2 per cent of total nitrogen in the form of bases precipitable by phospho-tungstic acid.

[1] KÜHNE and CHITTENDEN: *loc. cit.*, p. 452.

[2] SCHULZE, E.: Zeitschr. f. physiol. Chem., 1898, xxv, p. 360.

Hemialbumoses (G)

Total nitrogen in the fluid	1.3917 grams.
Nitrogen in phospho-tungstic acid precipitate	0.4687 "
Nitrogen in the form of ammonia	0.0805 "
Basic nitrogen, organic	0.3882 "

27.9 per cent of total nitrogen in the form of bases precipitable by phospho-tungstic acid.

Hemipeptone (I)

Total nitrogen in the fluid	1.3770 grams.
Nitrogen in phospho-tungstic acid precipitate	0.5492 "
Nitrogen in the form of ammonia	0.2637 "
Basic nitrogen, organic	0.2855 "

20.7 per cent of total nitrogen in the form of bases precipitable by phospho-tungstic acid.

From these results we see that antialbumid differs only quantitatively from the hemi bodies in yielding nitrogenous organic bases on decomposition with a mineral acid; a fact which affords additional evidence that the difference between the so-called hemi and anti bodies is not due to the lack of specific groups or radicles in the anti body, but rather to some difference in the arrangement of the groups or the state of combination by which the latter body is rendered resistant to the enzyme trypsin. Particularly noteworthy is the large proportion of nitrogen split off from hemipeptone in the form of ammonia, over 19 per cent of the total nitrogen appearing in that form.

In order to ascertain how far the possible presence of these "hexone" bases in antipeptone may affect the physiological action of the latter substance, an experiment was tried with a dog of 2.5 kilos body-weight in which 25 c.c. of a solution containing 0.73 gram of the mixed bases (from the phospho-tungstic acid precipitate) in the form of acetates were injected intravenously. The substance injected contained 15.5 per cent of nitrogen when dry. As a result it was found that the clotting time of the blood was not noticeably affected, while arterial pressure quickly fell from 135 mm. to 50 mm. Hg., but returned to the normal within three minutes.

ON THE COURSE OF IMPULSES TO AND FROM THE CAT'S BLADDER.

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THE experimental study here reported was undertaken in the Laboratory of Physiology of the Harvard Medical School, under the direction of Prof. W. T. Porter. To him I owe much, both for useful suggestions and for guidance throughout the work. The original intention was to determine, if possible, the exact nature of the mechanism for the transmission of crossed impulses in the region of the reflex bladder centre, as has already been done [43] for the impulses reaching the diaphragm through the phrenic nerves. So much routine work has developed, however, that the present paper is published with no reference to the original theme. The course of the impulses, the demonstration of the existence of physiologically continuous paths, and the definition of their limits, are all that have been attempted.

THE PERIPHERAL MOTOR NERVES.

As a preliminary to the experiments of the present research the course of the peripheral nerves to the bladder was studied both by dissection and by experiment, in several cats. In these operations, and in all others which were performed, the animals were fully anæsthetized with ether. Nothing was found at variance with the general results already described in the earlier literature [1] of the subject.

[1] For the topography of the motor nerves of the bladder see: Valentin [1]; Budge [5] (p. 118); Gianuzzi [6]; Budge [7] (p. 14) [8 17]; Sokownin [20 21]; Nussbaum [22 24]; Mosso and Pellacani [26] (p. 300), [27] (p. 43); Nawrocki and Skabitschewsky [30] (pp.

Motor fibres were found in the II and III sacral roots, and in the nervus erigens, which sends large strands to the hypogastric plexus. The question as to whether fibres from the I or IV sacral root are also carried toward the bladder by the nervus erigens was necessarily disregarded, for in none of the experiments which were undertaken was it possible first to explore the spinal nerves for variations, although such variations are of frequent occurrence. To avoid error, therefore, the sacral motor supply was regarded as a unit, and when it was to be extirpated at its exit from the cord all the sacral nerves were cut.

The fibres of the sympathetic group are carried regularly in the III, IV, and V lumbar roots, but the strands leading the impulses from the sympathetic chain to the inferior mesenteric ganglion vary much in position. They are usually four in number on each side, but sometimes three and sometimes five were observed; of fifteen cats eight had four, four had three, and three had five. The cases in which each strand proceeds separately to the ganglion are rare; usually two or three unite at some point in their course, and sometimes a plexus is formed. Their origin from the sympathetic chain is also subject to some variation, but they arise always in the region of the III, IV, and V lumbar segments. From the inferior mesenteric ganglion the hypogastric nerves convey the impulses to the hypogastric plexus, but accessory hypogastrics, as Langley and Anderson [41] (p. 413) have mentioned, are of frequent occurrence. Whenever such were present they were treated as parts of the hypogastric nerve itself.

The accompanying drawing is made from the dissection of a special case, and the nerves of the right side only are plotted. Four strands run from the lumbar sympathetic chain to the inferior mesenteric ganglion. From the ganglion arises the right hypogastric nerve, which in its course gives off an accessory hypogastric. Three branches from the nervus erigens unite with these two nerves to form the pelvic, or hypogastric plexus, and from the plexus four terminal branches run to the bladder. The number of terminal branches is more usually three, as in the dog (Griffiths [44], p. 64).

338–339) [31]; Langley [32] (pp. xxxiii and xxiv): Sherrington [33] (pp. 640, 678); Langley [36] (p. 237); Langley and Anderson [41] (p. 412); Griffiths [44] (pp. 62–67); François–Franck [38 46]; Langley and Anderson [47] (p. 79),[48] (pp. 76, 77, 78, 81, and 84), and [49] (pp. 374–379); and Courtade and Guyon [50] (p. 623). (The number after each name refers to the list of references at the end of the paper.)

The larger ganglia of the hypogastric plexus and of the vesical plexus on the surface of the bladder are also shown.

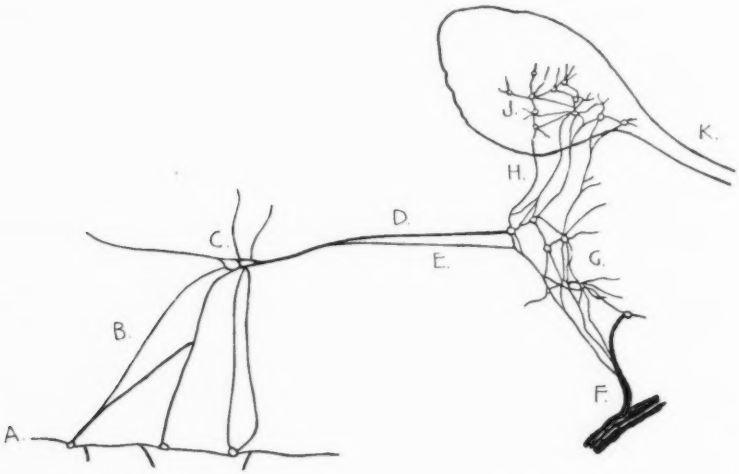

FIGURE 1. The vesico-motor nerves of the cat, on the right side, showing: A, the sympathetic chain, B, the rami to C, the inferior mesenteric ganglion, D, the hypogastric, and E, the accessory hypogastric nerve, F, the nervus erigens, G, the hypogastric plexus, H, the terminal nerves, J, the vesical plexus on the surface of the bladder, and K, the urethra.

It has been found impossible to demonstrate the presence in the sympathetic chain of vesico-motor fibres other than those carried in the paths described, that is, through the hypogastric nerves. Such fibres have been described by Gianuzzi [6] (p. 23), and Nussbaum [24]; while Sokownin,[21] and Nawrocki and Skabitchewsky [30] (p. 340, and Exp. III, p. 343) have failed to demonstrate their presence. Langley and Anderson [18] (p. 76) have obtained positive results in certain cases only. Though in some cases nerves which correspond to those described by Langley and Anderson (op. cit.) have been seen, they could not be proved to carry fibres having any control over movements of the bladder. Section of the hypogastric nerves left only the sacral paths open, and subsequent section of the sacral spinal nerves destroyed all possibility of producing bladder contractions by stimulating the cord. This was shown several times also incidentally in experiments undertaken for other purposes.

The Sensory Nerves of the Bladder, and the Reflexes.[1]

While the motor nerves may be explored by methods of direct stimulation with comparative ease, the demonstration of sensory nerves for the bladder is a matter of extreme difficulty. The methods are two in number. First, a nerve leading toward the bladder, or its central cut end, is stimulated while the animal is watched for respiratory or general body movements giving evidence of sensation. Second, the central cut end of a nerve suspected to contain sensory bladder fibres may be stimulated while the bladder is watched for contractions of reflex origin. Both methods are open to serious objections. Even if stimulation of the central cut end of a nerve trunk leading toward the bladder give evidence of having produced sensation, there is nothing to show that the sensory fibres stimulated belong to the bladder, unless the point of stimulation be between the bladder and the hypogastric plexus. Centrally of that point all motor nerves to the bladder are accompanied by similar nerves for the lower part of the intestine and for the genitals, and doubtless sensory nerves are combined in the same way. Again, it has been shown repeatedly that stimulation of almost any sensory nerve of the body will cause a reflex contraction of the bladder. Therefore such a reflex contraction produced by stimulating the central cut end of any nerve known to contain fibres for organs other than the bladder, cannot be taken as an index.

It has been stated (Sokownin[20]) that sensory nerves other than those belonging properly to the bladder have their reflex centre in the brain, while the bladder's own nerves produce reflexes from the vesical centre in the cord. If this were so, we should be provided with an easy means of distinguishing the true sensory nerves of the bladder; but this view is untenable. A reflex contraction of the bladder may be obtained by stimulating the central cut end of the sciatic nerve even immediately after section of the cervical or thoracic cord, as Mosso and Pellacani[26] (p. 292) pointed out. Nor is an injection of strychnine necessary for the demonstration of the reflex. It is so constant that it may be used to test the condition of

[1] The following references are of importance: Longet[4] (p. 614); Budge[7]; Paul Bert[13]; Gianuzzi[14,15]; Budge[17]; Goltz[18]; Sokownin[20,21]; Nussbaum[22-24]; Mosso and Pellacani[26,27]; Nawrocki and Skabitschewsky[31]; Langley[32]; Langley and Anderson[39,40,48]; Griffiths[44]; and Courtade and Guyon[50].

the spinal vesical centre, and as a means of determining the upper level of the centre.

It may be demonstrated, however, that the sacral nerves to the bladder contain sensory fibres. If the hypogastric nerves be cut to interrupt the connection with the lumbar cord, and if the peripheral bladder nerves be severed close to the bladder on one side, careful stimulation of their central divided ends will produce a reflex contraction. The path of this reflex is through the sacral nerves and through the cord, for if the sacral nerves of the opposite side be then cut, or if the lower lumbar or upper sacral cord be interrupted or destroyed, the reflex fails. If the first section of the experiment were made at any point in the course of the sacral nerves central to the hypogastric plexus, the reflex contraction produced by stimulating the central cut ends would be valueless. Doubtless the sensory nerves of the bladder are accompanied from that point by afferent nerves from other organs.

If the sacral nerves, thus shown to contain sensory nerves for the bladder, be cut on both sides, stimulation of their central cut ends produces no effect upon the bladder even though the hypogastric nerves are left intact. The hypogastric nerves do not carry motor impulses aroused by stimulating any of the afferent fibres contained in the sacral nerves. The sacral nerves are therefore both afferent and efferent for the reflex described.

It may be shown in a similar way that the hypogastric nerves contain afferent bladder fibres. If the terminal nerves to the bladder be cut on one side, and if the sacral supply to the hypogastric plexus be severed on both sides, stimulation of the central divided end of the peripheral nerves gives a well marked contraction of the bladder. This may be produced in either one of two ways. It may be a reflex from the inferior mesenteric ganglion, or it may be a reflex from the cord.

If now the connections of the inferior mesenteric ganglion with the cord be severed, stimulation of the central end of the divided peripheral nerves produces the reflex contraction as before. Such a reflex from the inferior mesenteric ganglion has already been demonstrated many times[1] by stimulating the central cut end of one hypogastric nerve, with the inferior mesenteric ganglion isolated. But the reflex obtained in this way might be mediated by afferent nerves from

[1] See Sokownin [20], Nussbaum [22 24], Nawrocki and Skabitschewsky [31] (p. 156), Langley and Anderson [39] and [41] (p. 415).

other organs supplied by the hypogastric nerves, and cannot be taken as demonstrating the presence of sensory fibres for the bladder in the hypogastrics. The point of stimulation must be peripheral to the hypogastric plexus. That the reflex centre is not in the cord may be shown in an animal in which the whole nerve supply to the bladder is intact. Stimulation of the central cut ends of the branches which unite the sympathetic chain with the inferior mesenteric ganglion produces no contraction of the bladder.

Sensory fibres are present, then, in the sacral supply to the bladder, and in the hypogastric nerves. Failure to demonstrate them between the inferior mesenteric ganglion and the cord, agrees well with three previous observations: Mosso and Pellacani [26] (p. 302) found that the bladder lost neither in sensation nor in motion after extirpation of the sympathetic rami to the ganglion; Langley and Anderson [40] (p. 188) have pointed out that the relative number of afferent to efferent fibres is smaller in the sympathetic rami than in the hypogastrics; and Griffiths [41] (p. 76) has found the effect of stimulating the central cut end of the hypogastric nerve upon the central nervous system to be wanting in experiments where similar stimulation of the sacral nerves has produced profound disturbances.

There is still another reflex produced by what we know to be sensory bladder nerves. Griffiths [41] (p. 78) has described what he considers probably a genuine reflex from the cells in the hypogastric plexus. On isolating this plexus and stimulating the central cut end of one of the three terminal branches to the bladder a contraction results. This has been repeatedly confirmed in the present research. The reflex may be obtained with electrical stimuli, with the mechanical stimulus of pinching, and with stimulation by means of a hot wire.

With regard to the reflexes produced by stimulation of sensory nerves other than those of the bladder, many observations have already been made.[1] Stimulation of the central cut end of the divided sciatic nerve produces a strong contraction of the bladder. If the sacral supply to the hypogastric plexus be cut on both sides the reflex fails altogether. None of the motor impulses from the reflex centre appears to be carried by the lumbar sympathetic nerves. Stimulation of the central cut end of the vagus in the neck was found ineffectual in all cases.[2]

[1] Paul Bert [18] (p. 650); Sokownin [20 21]; Nussbaum [22 24]; Mosso and Pellacani [26] (p. 291); Nawrocki and Skabitschewsky [31] (p. 155).

[2] For the literature upon this point the reader is referred to: Oehl [9] (p. 341),

The Position of the Reflex Centre in the Cord.

In determining the position of the centre in the cord from which reflex bladder contractions may be produced, no attempt was made to mark the lower level of the centre, no suitable method being devised. Its upper limit was determined by a method of sections, using the reflex obtained from the sciatic nerve as the test. The animal used was anæsthetized with ether, and a tube was placed in the trachea. The abdomen was opened in the linea alba, and in most cases the symphysis pubis was divided. A catheter was then passed up the urethra well into the bladder, and tied in place. By means of the catheter the bladder was partially emptied, and filled again with warm sodium chloride solution (0.9 per cent), through a side branch in a tube leading to a horizontal manometer. A shield, solid at the sides but made of wire netting at the upper end, was placed over the bladder and held in position by the body walls, which were drawn together and clamped. The animal was then turned back up, and the cord and sciatic nerves were exposed, the cord being kept under a constant stream of normal saline solution at body-temperature.

The sciatic nerves were then cut, and the central end of one of them was stimulated by means of an induced current strong enough to be distinctly felt on the tongue. An outward movement of the fluid in the manometer invariably followed. The cord was now cut across with a sharp knife and the reflexes were tested again. If the reflexes still persisted a second and lower cut was made. Sections above the level of the second lumbar root were always without effect, but any transverse section below the origin of the second lumbar root abolished the reflex.

In one series of these experiments the animal was anæsthetized, and the cord cut, from five to twenty-four hours before the final operation, the cat being kept under morphia in the intervening time. The purpose of this was to avoid any possible effect of shock upon the reaction of the reflex centre to stimulation through the sciatics. As before, the reflexes persisted after any section above the origin of the second lumbar root, and were abolished by cutting the cord anywhere below that point.

and [11], and Nussbaum,[22] who have reported positive results, and to Budge [7] (p. 11), Kehrer,[10] Paul Bert [13] (p. 651), Sokownin,[20 21] Nussbaum,[24] and Nawrocki and Skabitschewsky [31] (p. 255), for negative results.

The upper limit of the reflex bladder centre is then somewhere near the lower level of the origin of the second lumbar root. This does not agree with some of the results which have been obtained by former investigators. Budge's centre[5] (p. 118),[7] (p. 13), opposite the IV lumbar vertebra in the rabbit, was the point in the cord where irritability was greatest. But in many of the experiments of the present research it has been noticed, in work upon the lumbar cord, that after the cord ceases to conduct impulses at all, if the electrodes are brought in the region of the IV and V lumbar root origins, strong contractions of the bladder result. This has occurred even when the cord was destroyed by repeated longitudinal sections, so that the obvious explanation of Budge's results is that motor fibres were stimulated, not the cells in the centre. Masius[12] (p. 502), Gianuzzi,[14 15] Kupressow,[16] and Ott[23] (p. 61), place the centre for tonus of the vesical sphincter at about the level of the V lumbar root; but the whole bladder centre is undoubtedly of much larger extent in the cord. Gianuzzi[9] (p. 28) demonstrated two centres, the upper one opposite the III lumbar root; Sokownin,[20 21] places the centre opposite the IV lumbar; Nussbaum[22 24] describes the region of the III and IV lumbar roots; and Nawrocki and Skabitschewsky[31] (p. 155) give the II and V lumbar roots as the limits of the centre. These latter determinations all agree more or less closely with the one made in the present research.

The Effects of Stimulating the Peripheral Nerves.

The musculature of the bladder is of such a nature that the bladder may be considered as belonging to that group of the viscera in which the muscles are arranged in two layers, — longitudinal and circular. The longitudinal muscles, the detrusors, are more powerful in the wall of the viscus, while the circular muscles are strongest in the sphincter in the neck; thus a physiological separation might be sought for. Longet[4] (p. 614) indeed describes the sympathetic nerves as controlling the body of the bladder, while the sacral nerves control the sphincter. Von Zeissl[34 35] finds that the bladder conforms to von Basch's law of crossed innervation. The nervus erigens is described as motor for the body and inhibitory for the sphincter, while the hypogastric nerve closes the sphincter and inhibits the muscles of the body of the bladder. Courtade and Guyon[30] (pp. 625–626) also have given the hypogastrics and the sacral nerves as motor for the neck and the longitudinal muscles respectively. But against

these observations are those of Gianuzzi [9] (p. 24), Mosso and Pellacani [26] (p. 300),[27] (p. 43), Langley,[32] Sherrington [33] (p. 679), Griffiths [44] (p. 73), and Langley and Anderson,[48] who have found, in general, that the effect of stimulating the hypogastrics is different only in degree from that produced by stimulating the more powerfully motor sacral nerves.

Stimulation of the bladder, whether it be reflex or direct, and whether it be through the sacral or the sympathetic supply, produces a contraction which involves all parts of the musculature. Nothing has been observed which lends any support to the view that the innervation is crossed. There is, however, a difference in the effect of stimulating the hypogastric nerves, noted first by Langley [32], and later by Langley and Anderson [41 48] (p. 75), and Griffiths [41] (p. 74), according to whom stimulation often produces a dilatation, usually preceded by a short preliminary contraction.

Stimulation of the sympathetic supply at any point in its course produces a rise in the internal pressure of the bladder, followed usually by a fall of much greater extent than the original rise. The ratio may be as great as three to one. This is produced whether the lumbar spinal nerves, the rami to the inferior mesenteric ganglion, or the hypogastric nerves be stimulated, though more often one or two of the rami have an effect upon the bladder which is greater than that produced by the others. Several attempts were made to separate the fibres, stimulation of which produces the rise, from those which produce the fall, but they were always found to occur together anatomically. The injection of nicotine has in some instances seemed to affect the proportion between the rise and the fall, but no results of definite value were obtained, nor were any results obtained by the use of induction shocks, made to follow each other at varying intervals by using Ewald's interruptor.

Griffiths [41] (p. 74) found that stimulation of the hypogastric during the progress of contraction induced by stimulation of the pelvic splanchnic produced no result, an observation confirmed by Langley and Anderson [48] (p. 75), who found no satisfactory evidence of the presence in the hypogastrics of inhibitory fibres, nor any obvious inhibitory effect on the bladder when it had become contracted in consequence of exposure to the air. But very frequently in the course of the experiments here described it has been observed that when the bladder was insufficiently dilated to make the manometric variations apparent, stimulation of the hypogastric nerves was effec-

tual in producing a dilatation. Often warm normal saline solution was poured over the exposed surface of the bladder to produce the same effect, but the method of stimulation was nevertheless in common use.

Stimulation of the peripheral cut ends of any or all of the sympathetic rami to the inferior mesenteric ganglion on either side produces the same effect as stimulation of the nerves in their continuity. So also does stimulation of the inferior mesenteric ganglion with its spinal connections wholly or in part severed. If, however, the spinal rami, or the ganglion, be stimulated after section of one hypogastric nerve, then the bladder contracts only on the side with the intact hypogastric nerve. Similarly if the peripheral cut end of the divided hypogastric nerve be stimulated, contraction results only on the stimulated side, as noted by Langley and Anderson [11] (p. 423), and Griffiths [11] (p. 73). And again, the reflex contraction of the bladder produced by stimulating the central cut end of one hypogastric nerve involves only the side of the bladder supplied by the intact nerve.

The effect upon the internal pressure of the bladder of stimulating only one hypogastric nerve is not, however, the same as that produced by stimulation through both. In the latter case, as has already been stated, the manometer shows first a rise and then a fall. Stimulation through but one hypogastric causes the usual contraction of the corresponding half, and passive bulging of the other. When the stimulated side begins to relax in the typical way, the passive side contracts once more, and thus no great fall of internal pressure is recorded by the manometer.

In the case of the sacral nerve supply to the bladder stimulation of the sacral roots, of the nervus erigens, of the hypogastric plexus, or of the peripheral fibres, causes a strong contraction, followed by a slow return to the original form. Stimulation of the nerves in their continuity affects both sides of the bladder alike; but if the sacral supply be cut, and the peripheral end be stimulated, then contraction of only the corresponding half results. If the hypogastric plexus or the peripheral branches from the plexus to the bladder be cut in this way it is necessary to sever also the hypogastric nerve to prevent a reflex from the inferior mesenteric ganglion to the opposite half of the bladder. If, also, the central cut end of the divided sacral supply be stimulated, the resulting reflex contraction involves only the half of the bladder with the intact nerves. Gianuzzi [5] (p. 24), Sherrington [21] (p. 683), and Griffiths [11] (p. 73), have described the effect of stimula-

tion as unilateral; Langley and Anderson [48] (p. 80) do not agree with the earlier observations.

Relatively much stronger stimuli are required for the sympathetic nerves than for those of the sacral group. Even the strongest electrical stimulus applied to the sympathetic nerves will never produce so quick or so forcible a contraction as that commonly obtained from the sacral fibres. Mechanical stimulation, whether by pinching, ligating, cutting, or stretching the nerves, is effectual almost always; but the effect produced through the sacral nerves is always the greater.

Very frequently when the bladder is contracted, stimulation, even of the sacral nerve supply, produces relaxation, when the same stimulus would cause contraction if the bladder were at rest.

It has been observed repeatedly, especially on stimulating the sacral nerves, that the effect of a single stimulation is not a single contraction but a regular rhythmical series of contractions. Such a series is composed of from four to six oscillations, gradually diminishing in intensity.

There are differences in the persistence of irritability in the different regions of the peripheral nerve supply of the bladder, after the stoppage of the circulation. Nawrocki and Skabitschewsky [30] (p. 342) found irritability persisting in the hypogastrics for about an hour, in the sympathetic rami a shorter time. Usually the sympathetic rami cease to respond to stimulation almost immediately after the heart ceases to beat. In the hypogastrics irritability disappears after a very few minutes; but the bladder will still respond to stimulation of the nerves of its sacral supply sometimes for more than an hour. Since the differences depend doubtless on the fact that sympathetic cells die earlier than their fibres, as Langendorff [29] (p. 131) has pointed out, his method of determining the positions of the cells seems especially applicable to the study of the innervation of the bladder.

A constant possibility of error in the study of the effects of stimulating either sensory or motor bladder nerves is to be found in the rhythmic contractions normally present. These have been mentioned by Mosso and Pellacani [25] (p. 103), Ashdown [28] (p. 316), Sherrington [33] (p. 682), who showed that the rhythmic action arises in the wall of the bladder itself, and by Langley and Anderson [41] (p. 416), and Griffiths [45] (p. 254), who describe them as easily distinguished from those produced by stimulation. The rhythmical variations

in pressure which have been observed in the cat's bladder during the present research were not of such a nature as to seriously interfere with the experiments. In only one case was it necessary to abandon the experiment. Usually the amplitude of the variations is much slighter than that produced by stimulating any of the nerves. Sometimes the spontaneous contractions follow each other irregularly, but more often the rhythm is a regular one with intervals of from five to fifteen seconds. Ashdown[28] (p. 317) found the interval to be much longer. The opening of the body cavity had no constant effect either to increase or to abolish the rhythmical contractions.

The Course of Impulses in the Cord and in the Peripheral Nerves.

Budge[2] (p. 159),[17] (p. 310), and Valentin[3] (p. 327) showed that contractions of the bladder may be produced by stimulating the exposed cord. Nussbaum,[24] Mosso and Pellacani,[26, 27] Nawrocki and Skabitschewsky,[30] and Sherrington[42] (p. 71) have confirmed the original observations. Various observers have demonstrated the same effect upon stimulation of higher parts of the central nervous system, but the consideration of such results does not properly form a part of the present paper.

Bladder contractions may be produced by stimulating the cord at any point. The effect with both sets of peripheral motor nerves intact is the same as the effect produced by stimulating the nerves of the sacral group. If however the sacral nerves be excluded by section, then the effect is the same as that produced by stimulating the nerves of the lumbar group. Stimulation of the cord often causes an already contracted bladder to relax.

If the sympathetic rami to the inferior mesenteric ganglion be cut on one side, and if the sacral nerves be cut on the same side, stimulation of the cord still produces contraction through the uninjured nerves. If then a hemisection of the cord be made on the opposite side in the lower dorsal region, stimulation above the hemisection is still effective. The impulses are thus shown to cross somewhere in the cord below the level of the hemisection. It may be shown in the same way, by cutting the nerves and making the hemisection both on the same side, that the impulses which come down one side of the cord are able to produce contraction through the nerves of the same side.

Similarly, by excluding the nerves of each group separately, it can

be shown that impulses coming down one side of the cord are distributed to the sympathetic nerves of both sides, and to the sacral nerves of both sides. And it has also been shown that the reflex from the sciatic nerve crosses the cord and is effective upon the same side.

To determine the upper and lower limits of the crossing of impulses in the cord it is necessary only to split the cord in the median line until the crossing is stopped. The following protocol will give an example of such an experiment.

Cat XLIII, May 19, 1898. The animal was etherized and placed upon the operating board back down. A cannula was placed in the trachea, the abdomen opened in the median line, the symphysis pubis cut, and a catheter passed up the urethra, well into the bladder, and tied in position. The bladder was then filled with normal saline solution, warmed to body-temperature, and the catheter was connected with the tube of a horizontal manometer.

FIGURE 2. Diagram showing method of determining the lower level of the crossing of impulses in the cord.

Both hypogastrics were then cut, and also the sacral nerves to the hypogastric plexus on the left side. The bladder was shielded by a case, made partly of solid metal, partly of coarse wire netting, and the abdominal walls were drawn together and clamped. The animal was then turned over and the lower dorsal and the whole lumbar cord was exposed by removing the arches of the vertebræ. The cord was kept in a pool of normal saline solution, at body-temperature, from the time of the first exposure to the end of the experiment.

A right lateral hemisection of the cord was made at the XII dorsal root. Stimulation well above the hemisection by means of an induced current so weak as to be imperceptible on the tip of the tongue, resulted in a contraction of the bladder. Beginning at the hemisection (see Fig. 2) the cord was then split down the middle line one centimetre at a time, and after each new cut the effect of stimulating the dorsal cord was tested. In Figure 2 the black line indicating the complete section of the cord in the median line is broken to indicate the points at which each successive section joined the preceding one. When the cord was split to a point just below the lowest point of origin of the V lumbar root, stimulation was no longer effective. The electrodes were then placed on the right side of the cord immediately below the hemisection, and a well marked contraction of the bladder resulted.

In this way it was shown, in several experiments, that the lower limit of the crossing of impulses carried by the sacral roots is the lowest point of origin of the strands which make up the V lumbar root. In a series of experiments of a similar nature it was shown that the same point marks the lower limit of crossing of the impulses carried in the lumbar nerves, and of the impulses aroused reflexly by stimulating the central cut end of the divided sciatic. Each such experiment was at the same time used to prove that impulses also pass to the bladder uncrossed.

To determine the upper limit of the crossing of the impulses the experiment is of the same nature. The preliminary sections are the same, and the hemisection is made in the same place; but in this case the cord is split from below. The first cut is made well below the known lower limit of the crossing, and the splitting is continued up toward the hemisection until stimulation of the cord above the hemisection is no longer effective. The upper limit of the crossing of impulses in the cord was thus shown to be a point just below the origin of the strands which make up the II lumbar root, a point identical with that which was determined to be the upper level of the reflex centre. This determination was made separately for the impulses carried by the sympathetic fibres, for those carried by the sacral nerves, and for those aroused reflexly by stimulation of the sciatic nerves.

With the impulses carried by the lumbar nerves, however, a further determination was made. It has been shown by Langley and Anderson [40] (p. 188),[41] (p. 423),[48] (p. 73) that there is a crossing of impulses in the inferior mesenteric ganglion, — a fact easily demonstrated by cutting one hypogastric nerve and stimulating the peripheral cut ends of the sympathetic rami to the ganglion on each side separately. A series of experiments was therefore necessary to ascertain whether impulses crossing in the cord are identical with the ones which pass the inferior mesenteric ganglion uncrossed; or in other words, to determine whether the partial crossing in the inferior mesenteric ganglion bears any relation to the crossing of impulses in the lumbar cord. The accompanying figure (Fig. 3) illustrates diagrammatically the four possible paths open for impulses.

By making a right lateral hemisection of the lower dorsal cord, and stimulating only above the hemisection, impulses of the left side were isolated. The four possible paths, as shown in the figure, were then provided for by making sections between the cord and the

ganglion, and between the ganglion and the bladder, as indicated by the blocks in the figure. The sacral nerves were thrown out by a complete transverse section of the cord between the VI and VII lumbar roots. It was demonstrated that each of the four possible paths is taken. The crossing in the inferior mesenteric ganglion, therefore, cannot be regarded as supplementing the incomplete crossing of impulses which takes place in the cord.

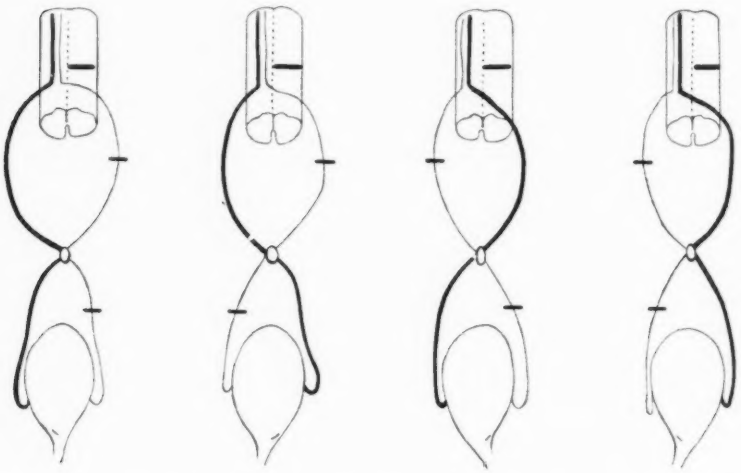

FIGURE 3. Diagram to illustrate the four possible paths open for impulses leaving the cord by the lumbar spinal roots, and passing through the rami to the inferior mesenteric ganglion, and through the hypogastric nerves to the bladder.

The experiments thus far have determined only the limits of the crossing of impulses which occurs below the XII dorsal root. The cord above that level still remains to be explored. It may be shown, by making a right lateral hemisection at the XII dorsal and a left lateral one at the first cervical root, and stimulating above the upper hemisection, that no impulses cross the median line between the two hemisections. In this experiment it is necessary to stimulate the cord by means of punctures, for the danger of spreading makes results obtained with electrical stimuli valueless. The effect of stimulation by punctures above the first cervical root before the hemisections are made is to produce a strong contraction of the bladder. After the first hemisection at the XII dorsal root the contraction is considerably

weaker; after the second hemisection at the first cervical root on the opposite side, no contraction at all can be obtained.

Above the first cervical root there is a certain variable amount of crossing. If the cord be hemisected anywhere in the cervical or dorsal regions, stimulation by puncture of the extreme upper part of the cord is effective on either side. If for example a right lateral hemisection be made at the first cervical root, then puncturing on either side above the hemisection produces contraction of the bladder. If the cord be then split from the first cervical root to the calamus scriptorius in the middle line, stimulation on the right side above the hemisection no longer produces any effect. This crossing between the medulla and the first cervical root is probably the lower part of a crossing which takes place in the decussation of the pyramids. It is of the same extent for impulses carried by the sympathetic fibres as for those carried by the sacral nerves.

With regard to the exact nature of the relation between the two halves of the spinal centre very little can be said. Griffiths[45] in referring to the centres of Budge and Gianuzzi, says: "I am inclined to go further, and even suggest that this centre is bilateral, each half controlling, as does each nerve, the corresponding half of the bladder; and that each half is connected by commissural fibres, so that under ordinary circumstances the two halves act in harmony with one another, and are influenced equally by stimuli transmitted to one or the other. The respiratory centre is just of such a nature. This view I have not yet subjected to experimental verification, but I hope soon to do so" (pp. 270 and 271).

The fact that the cord may be split in the middle line with only ordinary care, without interfering with any but crossed impulses, certainly shows that the vesical centre is distinctly bilateral. But that each half controls the corresponding half of the bladder is not true of impulses carried by the lumbar nerves. Each half of the centre controls both halves of the bladder by means of a crossing of impulses in the inferior mesenteric ganglion. Nor is there anything to show that the cells of one half of the centre do not send fibres through the sacral nerves of both sides.

The Path of the Impulses in the Cord.

Budge[2] in 1841 (p. 159) noted that stimulation of the anterior half of the cord was especially effective in producing bladder contractions, and in 1864[7] described an experiment in which the posterior

half of the cord was removed, stimulation of the anterior half producing contraction of the bladder (p. 15). Mosso and Pellacani [26] (p. 293),[27] (p. 36) demonstrated that the fibres for the bladder run in the posterior columns and in the posterior part of the lateral columns, and Ott [37] (p. 412) found that impulses pass to the bladder in the lateral columns.

Above the vesical centre it has been found that impulses pass down the cord in the posterior half of the lateral columns only. A right lateral hemisection of the cord was made and stimulation above the cut was shown to be effective. A cut was then made on the left side which severed the remainder of the dorsal half of the cord with the exception of the part occupied by the posterior columns. Stimulation above the point of partial section was then entirely without effect, while below the cut stimulation still produced a forcible bladder contraction. Again, the whole anterior half of the cord was cut without stopping the passage of impulses. In another case the middle third was severed, so as to interrupt the posterior and anterior columns, leaving the lateral ones intact, without interfering with the conduction of impulses. In still another experiment a complete section was made in the dorsal cord, and a portion five or six centimetres long, lying in a pool of warm saline solution, was split longitudinally in such a way that the dorsal half of the lateral column on one side was isolated. On tying a ligature around the upper end of this strip and raising it well above the pool in which the remainder of the cord still lay, stimulation produced marked bladder contractions.

Below the level of the centre the impulses were shown to pass in the lateral columns. If the lateral columns of the cord were cut at the V or VI lumbar root, leaving only the anterior and posterior columns intact, stimulation of the cord in the dorsal region failed to produce any effect after the lumbar nerves to the bladder were interrupted. And again, section of the middle third of the cord at the level of the VI lumbar root fails to interrupt the impulses carried by the sacral roots, or those aroused by stimulating the sciatic nerves. Since sections in the lower lumbar cord interfere more or less with the afferent fibres of the sciatic nerve, the fact that section of the lateral columns in that region will block motor impulses aroused reflexly by stimulating the sciatics was demonstrated in another way. The sacral supply to the hypogastric plexus was cut on the right side and the right sciatic nerve was stimulated. A crossed reflex was thus obtained. The lateral column of the left side was then cut at the VI

lumbar root, and the reflex contraction was abolished. The afferent elements in the reflex were thus left uninjured while the efferent fibres were shown to lie in the lateral columns.

Conclusions.

It has been shown, in confirmation of earlier work, that stimulation either electrically, mechanically, or thermally, of the motor nerve supply of the bladder, causes a contraction of the whole musculature of the viscus. Stimuli sufficient for the cord and for the sacral nerves are much weaker than those which must be used to stimulate the sympathetic supply. The irritability of the sacral nerves persists much longer than that of the hypogastrics after the heart ceases to beat, the sympathetic rami to the inferior mesenteric ganglion failing almost at once. The effect of stimulating the nerves of the sacral group is to cause a powerful contraction of the bladder, followed by a slow relaxation. The effect of stimulating the sympathetic nerves, on the other hand, is to produce a smaller contraction, followed in nearly every case by a dilatation of much greater extent. Impulses reaching the bladder through one hypogastric nerve, or through the sacral supply of one side, cause contraction of that half of the bladder only. There are sensory bladder nerves in the sacral supply, with reflex centres in the cord and in the hypogastric plexus. The sensory fibres for the bladder which pass up the hypogastric nerves have their reflex centre in the inferior mesenteric ganglion. Finally, the rhythmical contractions of the bladder are much smaller than those produced experimentally.

The further results described in the foregoing paper may be summed up as follows: —

The vesico-motor impulses passing down one side of the cord do not cross the median line of the cord below the first cervical root except in the region of the spinal vesical centre.

Impulses cross between the lower point of origin of the II lumbar root and the lower point of origin of the V lumbar; and there is a certain amount of crossing between the lower limit of the medulla and the first cervical root.

The crossing of impulses, identical in extent for those which leave by sacral or by sympathetic fibres, occurs between these same limits for impulses aroused reflexly by stimulating the sciatic nerves.

The upper limit of the reflex centre is the same as the upper limit of the crossing of the impulses.

The path in the cord of all vesico-motor impulses above the centre is in the posterior part of the lateral columns only. Below the centre the impulses pass in the lateral columns.

Impulses coming down one side of the cord pass out by the lumbar nerves of both sides, and by the sacral nerves of both sides.

Impulses leaving the cord by the lumbar roots of one side pass to the bladder by the hypogastric nerves of both sides. There is a second crossing of impulses in the inferior mesenteric ganglion, and all four possible paths are taken.

The motor impulses aroused reflexly by stimulating the sciatic nerve on one side pass out by the sacral nerves of both sides.

The vesical centre is bilateral, each half controlling both halves of the bladder at least through the lumbar nerves.

The motor fibres which carry the impulses aroused reflexly by stimulating the sacral sensory fibres lie wholly within the sacral nerves.

The motor fibres which convey the impulses aroused by stimulating the central cut end of the sciatic nerve, lie wholly in the sacral supply to the bladder.

The sympathetic rami to the ganglion have not been shown to contain any sensory fibres for the bladder.

The usual effect of stimulating the cord with all the peripheral nerves intact is the same as when the sacral nerves alone are stimulated; on excluding the sacral nerves the effect is the same as when the lumbar supply alone is stimulated.

A frequent effect of stimulation through the cord, and also through the peripheral nerves, is to produce dilatation of a previously contracted bladder.

Sometimes a single stimulation, lasting only long enough to cause a single contraction, gives rise to a rhythmical series of contractions or oscillations of gradually diminishing extent.

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ON SECRETORY NERVES TO THE SUPRARENAL CAPSULES.

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THE more exact study of the physiological effects of intravenous injections of extracts of the suprarenal glands marks the beginning of a new epoch in the history of these structures. These effects, studied and described independently and simultaneously by Cybulski and Szymonowicz[1] on the one hand and by Oliver and Schäfer[2] on the other, are now well known. In a normal animal, they consist in a pronounced slowing of the heart and a rise of blood pressure; in animals whose vagi have been thrown out of function either by section or by atropine, in heart acceleration and still greater rise of blood pressure.

The significance of the marked reactions exhibited by suprarenal extracts depends very largely upon the possibility of proving that the substance producing them is formed normally within the gland. It is conceivable, — indeed, it has been claimed,[3] — that the substance is formed in the dead gland as the result of post-mortem changes, and under the normal conditions of life does not exist. Cybulski first succeeded in demonstrating its origin in the living gland when he discovered that the blood collected from the adrenal vein gives a reaction similar to that of the substance itself, while blood from other veins does not possess this property.

Cybulski apparently made but few experiments of this kind. Langlois[4] succeeded in a single trial in repeating Cybulski's observation, while Oliver and Schäfer[5] state that in three attempts they

[1] CYBULSKI and SZYMONOWICZ: Gazeta Lekarska, 1895, p. 299; abstract in Jahresberichte der Thier-Chemie, 1895, p. 379: SZYMONOWICZ: Archiv f. d. ges. Physiol., 1896, lxiv, p. 97; CYBULSKI: Sitzungsber. d. Akad. d. Wissensch. in Krakau, 1895, March 4.

[2] OLIVER and SCHÄFER: Journal of physiology, 1895: also SCHÄFER: Textbook of physiology, 1898, i, p. 958.

[3] TIZZONI: quoted by LANGLOIS: Revue scientifique, 1897, p. 303.

[4] LANGLOIS: Revue scientifique, 1897, p. 303.

[5] OLIVER and SCHÄFER: *loc. cit.*; p. 275.

were unable to arrive at this result. In the spring of 1897 I undertook a series of experiments for the purpose of accumulating evidence on a point of such fundamental importance, and was soon convinced that Cybulski's observation was correct.[1] Further details of these experiments have been withheld for the reason that additional ones were in progress on a new point in the physiology of these same organs. Given the means of collecting the product of the normal activity of the gland in the blood issuing from its vein and of testing quantitatively the amount of the secretion in terms of characteristic physiological effects, the opportunity is presented of determining whether this secretory function is under nervous control, as the activity of the glands with external secretions is known to be. This question has but recently been raised with reference to the internal secretions, and its pursuit has yielded no result thus far for various reasons.

In the case of the suprarenal glands the conditions for investigating this point are peculiarly favorable, partly because of convenient anatomical relations, partly owing to the fact that here one has to deal with a secretion possessing specific reactions that can be readily recognized. It was this question of the existence of secretory nerves to the suprarenals to which my attention was from the first mainly directed, and the confirmation of Cybulski's discovery of the presence of the adrenal substance in the blood of its vein was simply a preliminary measure. The appearance of A. Biedl's[2] work has rendered the publication of the details of my first series of experiments unnecessary. In the second part of Biedl's paper he too confirmed the discovery of Cybulski, and then went on independently to investigate the influence of the nervous system on the internal secretion of the adrenals. As the result of stimulation of the splanchnic nerve in the thorax, he describes certain slight and rather vague morphological changes in the adrenal blood which seem to him to indicate the existence of secretory fibres. The experiments in which he compared the effects of injections of adrenal bloods collected before and during stimulation of the nerve gave only negative results, and he was compelled to acknowledge that his work had furnished no satisfactory evidence in support of this conclusion. My experiments on this point had been

[1] This result was reported to the physiological section at the meeting of the Congress of American Physicians and Surgeons held at Washington in 1897, brief notices of the report appearing subsequently in Science, 1897, p. 904, and in the Johns Hopkins University Circular, 1897, p. 49.

[2] Biedl, A: Archiv f. d. ges. Physiol., 1897, lxvii, p. 443.

practically concluded when Biedl's work came to my notice in the fall of 1897. As the method used by us was so similar in all essential respects and gave me results very different from his, I have since multiplied the number of my experiments in order to make the evidence as conclusive as possible. It is the result of these experiments bearing on the existence of secretory nerves to the suprarenal capsules that I desire to set forth briefly in the following pages.

Method.

The experiments involve two separate and distinct steps. The first consists in the collection of the blood to be tested: the second in the study of the effects of the intravenous injection of this blood into the same or into another animal. On account of their convenient size, dogs were used throughout. In every case special care was taken to have them completely anæsthetized with morphia and ether. To collect the adrenal blood, the abdomen was opened widely by means of generous longitudinal and transverse incisions, and the viscera immediately protected with sponges saturated with warm normal saline solution. Pushing the viscera over to the right, the left adrenal gland was exposed and the vein quickly prepared. A ligature was placed around the vein on either side of the gland, the one on the mesial side serving to tie off the central end of the vein, the other, on the lateral side, being used to tie in the cannula. This cannula was simply a straight glass tube about twelve centimetres long. When in position it lay horizontally and reached several centimetres beyond the body wall. The blood dropped from the free end of the cannula directly into the collecting vessels. The ligature on the central end of the vein prevented any reflux from the vena cava, and that around the neck of the cannula, being close to the gland, hindered the admixture of any blood from the numerous muscular branches joining the capsular vein. The blood issuing from the cannula was therefore derived from the gland alone. When the blood clotted in the cannula, and this occurred the more frequently the tardier the outflow, the clot was easily removed by introducing a fine straw. The splanchnic nerve was prepared from the point where it emerges from under the diaphragm to where it sends off its branches to the adrenal gland and renal plexus, and after placing a tight ligature around it, as high as possible, was arranged upon shielded electrodes. A kymographic record taken from the carotid artery served the twofold purpose of indicating the condition of the animal and of controlling the stimulation

of the nerve. The interrupted current from an ordinary induction coil was used to stimulate the nerve, and in a given stimulation period the current was alternately turned into and taken out of the nerve for approximately equal intervals of ten to fifteen seconds. The current was made strong enough to give a decided rise of blood pressure with each application; so long as this rise occurred it was taken for granted that the irritability of the nerve persisted and that any secretory fibres contained in it would respond.

The following specimens of blood were collected in every complete experiment: *a*, from the femoral vein; *b*, from the adrenal vein before stimulation; *c*, from the adrenal vein during stimulation. In a few cases the condition of the animal continued good enough to enable a number of additional specimens to be obtained. These included: *d*, from the adrenal vein after stimulation; *e*, from the adrenal vein during a second stimulation period; *f*, from the adrenal vein in the second interval, that is to say, in the period succeeding the second stimulation. The time consumed in collecting each specimen naturally varied with the rate of outflow. The object was to obtain sufficient quantities of each kind of blood for the subsequent injections; and as experience soon taught that 30 c.c. or more of blood were required for each dose to ensure positive results in dogs, enough time was allowed to collect 75 to 100 c.c. of each kind. The actual times required for this are given in the tabulated protocols of the experiments. They varied between fifteen and seventy minutes. It goes without saying that quantities of blood so large as these can be best obtained from large animals, and such were accordingly selected for this purpose. Small dogs, on the contrary, are better adapted for testing the reaction, since for a given volume of blood injected, the smaller the dog the larger relatively will be the dose of adrenal substance. The several lots of blood were defibrinated and filtered through muslin. It should be stated that the femoral blood taken previous to opening the abdomen was used in every experiment as a control upon the normal adrenal blood.

For the second part of the experiment a fresh dog likewise under morphia and ether, was generally used. By the time the necessary blood was obtained from the first dog, its general condition was unfavorable for good reactions. The animal was connected with the kymograph in the usual manner for a blood pressure record, and the bloods to be tested were injected into the external jugular vein at suitable intervals. In all the later experiments provision was made

to have the blood to be injected raised to as near the body-temperature as possible. Whenever the supply of blood permitted, the injections were made in two series, the first while the vagi remained intact, the second after double vagotomy or after the administration of five milligrams of atropine. At the end of the second series, normal salt solution in amounts equal to the doses of blood previously used was injected in order to eliminate any possible effect of the mere introduction of that amount of fluid into the circulation; and finally by means of adrenal extract it was determined whether the physiological mechanisms on which the reaction depends were still functional at that time.

Results.

As stated above, these experiments were intended to determine whether the activity of the suprarenal glands is under the influence of secretory nerves. The specific constituent of their secretion which for convenience may be spoken of provisionally as the "adrenal substance" until the chemists succeed in isolating and identifying it, we now know passes directly from the gland into the blood stream, and its presence there can usually be demonstrated with ease by collecting the blood as it flows from the gland vein and injecting it into the same or another animal. In dogs 10 c.c. of adrenal blood or even less may at times suffice for this demonstration; but the result is more certain with 30 c.c. or more. Whenever the amount is large enough, and this naturally depends on the concentration in the active principle, the result will be a change in heart-rate and blood pressure similar to that produced by extracts of the gland. If the suprarenal glands are supplied with secretory fibres whose stimulation augments the production of adrenal substance, evidence of the same should be furnished in the increased concentration of the blood attendant upon their stimulation, and in the correspondingly heightened effects of this blood upon the heart and blood vessels. The nervous connections of the suprarenal glands in the dog have been figured and described by Jacobi.[1] According to him the suprarenals might obtain secretory fibres from several sources, seeing that they are intimately connected with the solar and renal plexuses as well as with the splanchnic nerve. In my experiments the latter nerve alone was stimulated to obtain secretory effects upon the gland. Figures 1 and 2 will serve as illustrations of the result obtained. Figure 1 presents two blood pressure

[1] Jacobi: Arch. f. exper. Pathol. u. Pharmakol., 1891, p. 171.

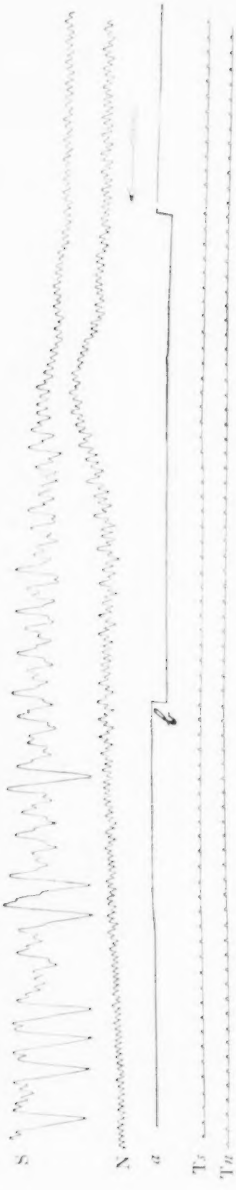

FIGURE 1. One third original size. To be read from right to left. The effect on the blood pressure in a dog with intact vagi of injecting 35 c.c. of blood taken from the adrenal vein of another dog before (curve N) and during (S) stimulation of the splanchnic nerve. An interval of ten minutes separates the two curves. The break (*b*) in the line *a* measures the duration of injection (32 sec.). *Tn* and *Ts* are the base-lines of curves *N* and *S*, respectively; they also give the time in seconds.

tracings from a dog with intact vagus nerves taken at an interval of about ten minutes. N shows the effect of 35 c.c. normal adrenal blood, S that of the same amount of stimulation blood. The blood in both cases was injected slowly into the external jugular vein at body-temperature, the injection occupying 32 seconds, as indicated by the break (*b*) in the line *a*. Each blood pressure curve is accompanied by its own base-line, which also gives the time in seconds. T*n* belongs to the curve N, T*s* to S. These records are part of Experiment V, May 20, 1898. Figure 2 gives similar records from Experiment VIII, May 31, 1898. In this case the amount of each kind of blood was 65 c.c. and the injections were made after the dog had received a full dose of atropine. The vagi were accordingly completely paralyzed, and the effects consist in rise of blood pressure and acceleration. As in Fig. 1, the curve S is the record for the stimulation blood, N that for normal blood, *a* gives the time and duration of the injection, while T*s* and T*n* are the respective base-lines. These figures show plainly that a given bulk of adrenal blood taken during stimulation has a decidedly greater effect than the same bulk of normal adrenal blood, meaning by "normal" blood that which was taken when not stimulating. With the vagi intact in the

reaction dog, this augmented effect is particularly manifest in the more pronounced inhibition of the heart (see Fig. 1). In atropinized dogs, the stimulation blood gives a greater rise of blood pressure and more extensive acceleration.

Biedl[1] has shown, however, that the splanchnic nerve contains vasodilator fibres for the adrenal, so that stimulation of its trunk must materially modify its blood-supply. Before the increased efficacy of the blood noted above as resulting from stimulation of this nerve can be interpreted as indicating the existence of secretory nerves, these accompanying vasomotor disturbances and their possible influence on the gland must be taken into account. To determine the value of this factor so far as my result is concerned, I have arranged my results in tabular form (page 210) so as to show side by side with the effect of any given specimen of blood the condition of the circulation prevailing at the time of its collection. It will serve practically as a brief protocol of the several experiments.

The injections were made in dogs in which the vagi were intact or in which the inhibitory influence of these nerves had

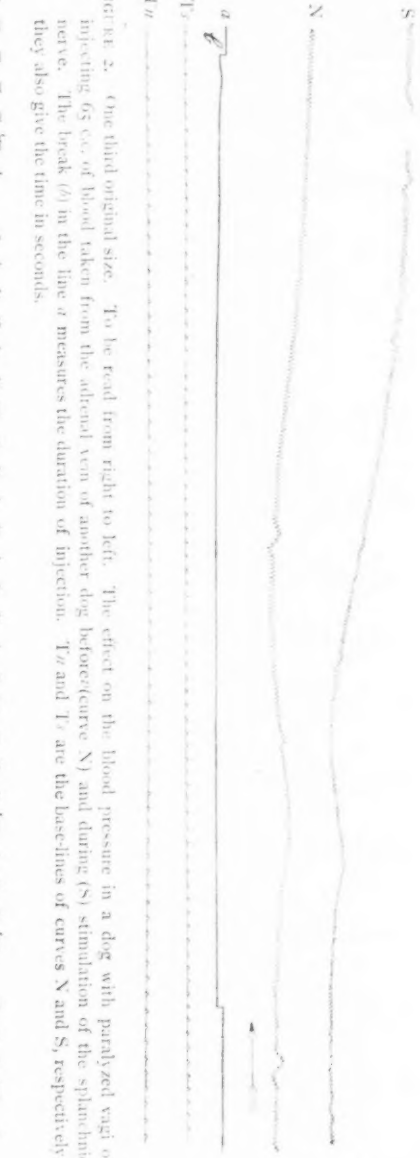

FIGURE 2. One third original size. To be read from right to left. The effect on the blood pressure in a dog with paralyzed vagi of injecting 65 c.c. of blood taken from the adrenal vein of another dog before (curve N) and during (S) stimulation of the splanchnic nerve. The break (*b*) in the line *a* measures the duration of injection. *Tn* and *T* are the base-lines of curves N and S, respectively; they also give the time in seconds.

[1] BIEDL: Archiv f. d. ges. Physiol., 1897, lxvii, p. 443.

1 No. of Exp.	2 Condition of Vagi.	3 Amount and character of injections.								4 Diminution (−) or increase (+) in the Heart-rate per minute.							
		a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*
I	Intact	25	25	25	....	....	....	....	....	−57	−48	−36	....	....	....	....	....
	Atropine	30	35	25	....	....	....	....	10	−16	−20	−16	....	....	....	....	−18
II	Atropine	35	40	40	....	....	....	....	10	− 9	+ 8	+63	....	....	....	....	+48
III	Cut	16	38	45	....	....	....	....	5	−15	0	+ 6	....	....	....	....	+33
		32°	35°	32°.5	....	....	....	....	....	....	....	....	....	....	....	....	....
IV	Intact	35	35	35	....	....	....	....	....	−29	−14	−32	....	....	....	....	....
		38°	39°	39°	....	....	....	....	....	....	....	....	....	....	....	....	....
V	Intact	35	35	35	....	....	....	....	....	+15	−18	−36	....	....	....	....	....
Fig. 1		39°	38°.5	38.°5	....	....	....	....	....	....	....	....	....	....	....	....	....
	Atropine	35	35	35	30	....	....	35	20	+ 8	+66	+66	+45	....	....	0	+72
		38°	39°	39°	40°	....	....	39°	39°	....	....	....	....	....	....	....	....
VI	Intact	50	50	50	50	50	50	....	....	−12	−12	−24	−14	−20	−16	....	....
		39°	39°	38°	38°.5	38°	39°	....	....	....	....	....	....	....	....	....	....
	Atropine	50	50	50	50	50	50	50	50	0	0	+18	0	+13	+ 9	0	+51
		39°.5	39°.5	39°	38°	38°.5	39°	39°	39°.5	....	....	....	....	....	....	....	....
VII	Atropine	35	40	40	35	35	....	40	25	0	0	0	0	+28	....	0	+51
		39°	39°	39°	38°.5	39°	....	39°.5	....	....	....	....	....	....	....	....	....
VIII	Atropine	65	65	65	....	....	....	65	20	−10	+23	+36	....	....	....	0	+80
Fig. 2		39°.5	39	39	....	....	....	39°.5	....	....	....	....	....	....	....	....	....

5 — Diminution (−) or increase (+) in Blood Pressure, in millimetres of mercury.								6 — General Data.
a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	
+ 7	+10	+21	....	....	....	....	....	Stimulation of Splanchnic caused rise of blood pressure and acceleration of outflow from adrenal vein.
+10	+22	+36	....	....	....	....	+ 95	Blood collected included —
								a 60 c.c.
								b 65 c.c. in 25 min. Aver. rate = 156 c.c. per hr.
								c 80 c.c. " 70 " " " 68 c.c. "
+10	+ 4	+53	....	....	....	....	+ 63	Stimulation of Splanchnic gave same effect as in Exper. I.
								Blood collected included —
								a 65 c.c.
								b 75 c.c. in 30 min. Aver. rate = 150 c.c. per hr.
								c 105 c.c. " 55 " " " 120 c.c. "
+ 8	+20	+41	....	....	....	....	+ 56	Stimulation of Splanchnic same as above.
....	....	....	....	....	....	....	....	Blood collected included —
								a 90 c.c.
								b 70 c.c. in 30 min. Aver. rate = 140 c.c. per hr.
								c 85 c.c. " 30 " " " 170 c.c. "
0	+ 7	+26	....	....	....	....	....	Stimulation of Splanchnic as above.
....	....	....	....	....	....	....	....	Blood collected included —
								a 50 c.c.
								b 50 c.c. in 30 min. Aver. rate = 100 c.c. per hr.
								c 75 c.c. " 30 " " " 150 c.c. "
+19	+11	+50	....	....	....	....	....	Stimulation of Splanchnic as before.
....	....	....	....	....	....	....	....	Blood collected included —
0	+58	+61	+54	....	....	+ 6	+ 80	*a* 85 c.c.
....	....	....	....	....	....	....	....	*b* 100 c.c. in 40 min. Aver. rate = 150 c.c. per hr.
								c 75 c.c. " 45 " " " 100 c.c. "
								d 30 c.c. " 50 " " " 36 c.c. "
+13	+ 6	+13	+ 7	+14	+ 7	....	....	Stimulation of Splanchnic as before.
....	....	....	....	....	....	....	....	Blood collected included —
+10	+12	+41	+13	+35	+30	+ 4	+104	*a* 135 c.c.
....	....	....	....	....	....	....	....	*b* 165 c.c. in 23 min. Aver. rate = 432 c.c. per hr.
								c 125 c.c. " 18 " " " 420 c.c. "
								d 100 c.c. " 15 " " " 400 c.c. "
								e 135 c.c. " 20 " " " 405 c.c. "
								f 125 c.c. " 60 " " " 125 c.c. "
+ 4	+10	+12	+ 4	+34	....	0	+ 66	Stimulation of Splanchnic as before.
....	....	....	....	....	....	....	....	Blood collected included —
								a 100 c.c.
								b 145 c.c. in 35 min. Aver. rate = 248 c.c. per hr.
								c 110 c.c. " 25 " " " 264 c.c. "
								d 60 c.c. " 30 " " " 120 c.c. "
								e 35 c.c. " 20 " " " 105 c.c. "
+24	+22	+68	....	....	....	+ 6	+161	Stimulation of Splanchnic as before.
....	....	....	....	....	....	....	....	Blood collected included —
								a 75 c.c.
								b 90 c.c. in 35 min. Aver. rate = 155 c.c. per hr.
								c 100 c.c. " 30 " " " 200 c.c. "

been abolished either by section or by poisoning with atropine (Column 2, page 210). Wherever atropine was employed it is to be understood that the drug was given in sufficient quantity to paralyze completely the inhibitory fibres before the injections to be tested were made.

In Exp. I the reaction dog furnished the blood used for injection; in the remaining experiments the blood in each instance was drawn from another animal.

It will be observed that Columns 3, 4, and 5 are each subdivided into eight subsidiary ones, headed respectively by the first eight letters of the alphabet. These letters have the same significance wherever they occur in this paper, and refer in each case to a blood-specimen or other reagent tested on the reaction dog. *a*, means blood from the femoral vein; *b*, blood from the adrenal vein previous to stimulation of the splanchnic nerve; *c*, blood from the adrenal vein during stimulation or, briefly, 1st stimulation blood; *d*, blood from the adrenal vein after the first stimulation period or, briefly, 1st interval blood; *e*, blood from the adrenal vein during a second stimulation period, or 2d stimulation blood; *f*, blood from the adrenal vein during the 2d interval; *g*, normal saline solution; *h*, extract of adrenal gland of no definite concentration, the figures giving simply the volume of the injection. In Column 3 the temperature of the injection is indicated by figures standing immediately beneath those giving the volume; where no temperature is given, it is to be understood that the injection was made at room temperature. The figures in Column 4 give the changes in the heart-rate per minute resulting from the injection. A minus sign in front of a number indicates inhibition; a plus sign, acceleration. The blood pressure changes (Column 5) are recorded in millimetres of mercury, the minus sign here signifying a fall of blood pressure; a plus sign, rise of pressure for so many millimetres of mercury. In respect to both heart-rate and blood pressure the figures give the maximal effect for the injection, and were obtained by taking the algebraic difference between the pulse-rate and blood pressure for the ten second period just preceding an injection, and the pulse-rate and blood pressure, respectively, for that ten second interval after the injection when the effect was greatest. Lastly, Column 6 furnishes certain miscellaneous general data of more or less importance to the correct interpretation of the result. The remarks being written out in full will explain themselves.

Effects of "b" and "c" compared, when injected into dogs with intact vagi. — Owing to the lack of sufficient quantities of blood, it frequently happened that only a single series of injections could be made. In such cases, they were preferably made into dogs whose vagi had been thrown out of function, as it seemed to me that there was a little more certainty of detecting and estimating small differences in the blood pressure changes under these circumstances than when complicated by inhibitory effects on the heart. For this reason the table contains only four comparative tests on dogs with intact vagus nerves. These occur in Experiments I, IV, V, and VI. While the doses of normal and stimulation blood in each case were equal, it will be seen that the stimulation blood throughout gave the greater rise of pressure and except in Experiment I a greater inhibition. The difference in favor of the stimulation blood is moreover well-marked, ranging from one hundred per cent upwards. Thus, in

Exp. I.	25 c.c. *b*	slowed heart	48	per min.	and raised	pressure	10	mm. Hg.
	25 c.c. *c*	"	36	"	"	"	21	"
" IV.	35 c.c. *b*	"	14	"	"	"	7	"
	35 c.c. *c*	"	32	"	"	"	26	"
" V.	35 c.c. *b*	"	18	"	"	"	11	"
	35 c.c. *c*	"	36	"	"	"	50	"
" VI.	50 c.c. *b*	"	12	"	"	"	6	"
	50 c.c. *c*	"	24	"	"	"	13	"

The exceptional result so far as the heart-rate is concerned in Exp. I is, in all probability, accounted for by the fact that in this case the blood was injected at room temperature. This being considerably below that of the dog (approximately 20° C.), it is evident that the direct effect of the low temperature on the heart must enter as a disturbing factor, the extent of this influence varying with the rapidity of the injection. That this is the correct explanation is further indicated by the fact that the vaso-constriction and the resultant rise of blood pressure, upon which the temperature difference could have no effect, was even in this case greater for the stimulation blood than for the normal. The unusually marked slowing of the heart observed in this experiment must therefore be regarded as the summated effect of cold and of adrenal substance cooperating to produce a change in the same direction. Compare also the results given below obtained on the same dog after the exhibition of atropine.

Effects of "b" and "c" compared, when injected into dogs after section or complete paralysis of both vagi. — The number of observations of this class was considerably larger, but the results were equally uniform and in the same sense. Thus, in

Exp. I.	35 c.c. *b*	slowed heart	20	per min. and raised pressure			22	mm. Hg.
	25 c.c. *c*	"	16	"	"	"	36	"
" II.	40 c.c. *b*	quickened heart	8	per min. and raised press.			4	"
	40 c.c. *c*	"	63	"	"	"	53	"
" III.	38 c.c. *b*	"	0	"	"	"	20	"
	45 c.c. *c*	"	6	"	"	"	41	"
" V.	35 c.c. *b*	"	66	"	"	"	58	"
	35 c.c. *c*	"	66	"	"	"	61	"
	30 c.c. *d*	"	45	"	"	"	54	"
" VI.	50 c.c. *b*	"	0	"	"	"	12	"
	50 c.c. *c*	"	18	"	"	"	41	"
	50 c.c. *d*	"	0	"	"	"	13	"
	50 c.c. *e*	"	13	"	"	"	35	"
	50 c.c. *f*	"	9	"	"	"	30	"
" VII.	40 c.c. *b*	"	0	"	"	"	10	"
	40 c.c. *c*	"	0	"	"	"	12	"
	35 c.c. *d*	"	0	"	"	"	4	"
	35 c.c. *e*	"	28	"	"	"	34	"
" VIII.	65 c.c. *b*	"	23	"	"	"	22	"
	65 c.c. *c*	"	36	"	"	"	68	"

In this series Experiment I again presents the only exception in that it gives a slowing of the heart for both normal and stimulation blood where acceleration should occur. This result, however, only confirms what was said above concerning the part played by the low temperature at which the blood in this experiment was injected. The retarding influence of the cold acting on the heart directly simply over-compensated the accelerating tendency of the adrenal blood. In the remaining experiments the stimulation blood either equalled or surpassed the normal blood both as to the effect on the heart and on the peripheral vessels. Experiment VI furnishes a particularly interesting and striking illustration of the influence which stimulation of the splanchnic nerve has on the content of adrenal substance in the blood. In this experiment I was able to continue the collection of blood

through five alternate periods of rest and stimulation, obtaining altogether 650 c.c. of adrenal blood. The table shows a distinct increase in the concentration of the blood during each stimulation period, followed by a diminution in the intervening periods of rest. In Experiment VII a similar series was obtained; but the concentration being low throughout, the result is less conspicuous. In this connection it may be interesting to call attention to the extended variation in the concentration as regards adrenal substance observed in normal adrenal blood from different individuals of the same species. From those in which so much as 40 and 50 c.c. of blood gave no acceleration and only 10 and 12 mm. rise of pressure respectively, they range in my table to such as gave an acceleration of 66 beats per minute and 58 mm. rise of pressure with 35 c.c. of blood. In fact, I often met with instances in which 10 c.c. still gave a distinct reaction, as recorded also by others engaged upon this problem. In these individuals with exceptionally high concentration of the normal adrenal blood, stimulation of the nerve failed to produce the usual increase, suggesting the idea that in them the gland was already at the maximum height of secretion. Nothing can be stated at present concerning the normal mechanism through which this secretion is regulated; but every analogy would lead us to expect these very fluctuations in an apparatus of such a seemingly important function. It seems to me also that these normal variations may account for the negative result obtained by Oliver and Schäfer. They probably met with slowly secreting glands, and used an insufficient amount of the relatively dilute blood. In my experiments, in which comparatively large volumes of blood were used, the adrenal effect was never completely absent, however small it may have been in several instances. For example, in Experiments VI and VII, in which the volumes used were respectively 50 and 40 c.c., there was still a rise of 12 and 10 mm. in the blood pressure, while the heart-rate continued unchanged. In spite of the slight extent of this change, it must be regarded as a genuine adrenal effect because the same volumes of femoral vein blood had given still smaller changes just before, and normal salt solution given directly after gave in one case (VI) only 4 mm. rise and in the other (VII) no rise whatever. The result was therefore not due to the mere mechanical effect of that much fluid added to the circulation.

Relation between blood-supply and rate of secretion in the suprarenals.— I have shown in the preceding sections that stimulation

of the splanchnic nerve augments adrenal secretion. A point of great interest arises here as to the interpretation to be placed upon this fact. Is the augmentation to be ascribed to a direct influence of the nerve on the metabolism of the secreting cells, or is it indirectly produced by the accompanying vascular phenomena? Increased functional activity is commonly associated with vascular dilatation and increased blood-flow, and in some organs, for example the kidneys, the vascular change is the direct cause of the functional variation. Does a similar relation obtain for the suprarenals? This is a legitimate question, and if the results showed a regular variation in the outflow from the adrenal vein accompanying the stimulation it would be difficult to reach a conclusion.

It is well known that stimulation of the splanchnic nerve does produce extensive vasomotor changes. In my experiments the customary rise of general arterial pressure produced by such stimulation was regularly obtained. In fact, as previously stated, this reaction was relied upon to indicate the maintenance of irritability in the nerve. This general rise of pressure in itself would tend to increase the blood-supply to the adrenal, provided its intrinsic arteries did not participitate in the constriction. Far from being constricted Biedl[1] has shown that they actively dilate on account of the vasodilator fibres supplied to the gland in the splanchnic trunk. Local dilatation simultaneous with general constriction constitute the most favorable condition for increasing the blood-supply to an organ, and one should expect the outflow from the adrenal vein to show a marked acceleration in the stimulation periods. As a matter of fact Table I presents examples of every possible relation between the rate of outflow and the increased functional activity attendant upon stimulation of the nerve. The average outflow in a given stimulation period was sometimes the same as in the preceding period of rest, and when it was changed, the change was as frequently retardation as acceleration. The reason for this is not far to seek. It must be remembered that the conditions of the experiment necessarily induce a steady decline of blood pressure in the dog from which the blood is being collected. This follows not only from the extensive abstraction of blood (ranging from 150 c.c. to over 700 c.c.) but is due also in part to the unavoidable partial exposure of the abdominal viscera with its accompanying fall of temperature and increasing vascular paralysis. In two successive half-hour periods, therefore, regularly less blood should be ob-

[1] Biedl: Archiv f. d. ges. Physiol., 1897, lxviii, p. 443.

tained in the second than in the first, independently of any incidental stimulation. Together with this fact must be considered the manner of stimulation employed in these experiments. A so-called stimulation period extending over 18 to 70 minutes embraces alternating 10 to 15 second intervals of actual stimulation and rest. With each application of the current, the outflow is decidedly accelerated, frequently as much as one hundred per cent. But it drops back just as quickly in the succeeding interval of rest, and the normal rate to which it returns constantly diminishes more and more. The average outflow for the whole period, which must be taken as the measure of the blood-supply during that time, is the resultant of two factors whose influence tends in opposite directions. The actual result in a given experiment depends on their relative potency, and so it happens that in one case an increase, in a second a decrease, in a third no change is observed. In passing I may refer also to a third factor which occasionally comes into play, and that is the mechanical interference to the outflow from the development of blood-clots in the vein and cannula. This is especially apt to occur when the outflow is slow, and can be largely avoided by the use of large dogs, as recommended above in another connection.

Conclusion.

The recent advances in our knowledge of the various glandular organs, especially in that of the several ductless glands, by which a new chapter on "Internal Secretion" has been added to physiology, naturally led to the inquiry as to the dependence of this new function upon specific nerves. The idea of secretory nerves is so familiar in the physiology of glands with an external secretion, that it could not fail to suggest itself in connection with those producing internal secretions. Biedl gives the references to the literature bearing on this point, and shows that as early as 1889 Wyss[1] raised this question with reference to the thyroids. The conception is therefore by no means a new one, although all the credit which he demands should be conceded to Biedl for bringing it prominently to the front. Hitherto no proof for the existence of secretory nerves to glands with an internal secretion has been obtained. So far as the adrenals are concerned, Biedl alone has investigated the point, and with what result may best be stated in his own words. He says, on page 480: "My observations have not yet enabled me to reach the final solu-

[1] Wyss, A.: Correspondenzblatt fur Schweizer Aerzte, 1889, no. 6.

tion of this problem. It appeared that the blood drawn from the vein during stimulation has no less, or at least no significantly less effect than that drawn previous to stimulation. An increased action was not present." And again, on page 481: "I am fully aware that I was not in a position to bring forward exact evidence for these statements."[1]

My results recorded in the preceding pages furnish the first demonstration of the actual existence of nerves for controlling an internal secretion. Starting with the fact first discovered by Cybulski and now abundantly confirmed by Langlois, Biedl, and myself, that the internal secretory products of the suprarenals pass directly into the blood stream, it has been shown in the first place that the amount of the active substance can be increased by stimulating the splanchnic nerve below the diaphragm. This conclusion necessarily follows from the fact that the blood from the adrenal vein collected during stimulation of this nerve produces the characteristic adrenal reaction with greater intensity than the blood taken before stimulation, comparing them volume for volume. It has been stated by Biedl[1] on *a priori* grounds that the stimulation blood might be poorer in adrenal substance in spite of heightened activity of the gland because of its more rapid passage through the organ. The same author is also responsible for the statement that with repeated injections of adrenal blood of the same concentration the reaction becomes weaker and weaker. I have made no attempt to verify this last statement, as I regularly injected the stimulation blood after the normal. The stronger reaction with the former must therefore *a fortiori* signify increased concentration if Biedl is right.

Secondly, it has been shown that this effect of splanchnic stimulation can in no way be referred to the indirect effect on the blood-flow through the gland. The increased secretion is observed whether the outflow from the vein increases, decreases, or remains constant. Under these circumstances it is not justifiable to suppose that a causal relation can obtain between the phenomena heightened activity of the gland and rate of blood-flow, and the conclusion that the splanchnic supplies true secretory fibres to the adrenal is extremely probable. The proof is as strong as the method employed is capable of yielding, especially when the number of the experiments and the uniformity of the results are considered. We must look to other methods for corroboration, and perhaps a repetition and extension of

[1] Biedl: *loc. cit.*, p. 443.

the tests with suitable pharmacological agents will be attended with better success than has been obtained with them hitherto.

Summary of Results.

1. The active principle of adrenal extract is present in the blood collected from the adrenal vein and constitutes therefore a true internal secretion.

2. The amount of adrenal substance in the adrenal blood as measured by the extent of the physiological effects it is capable of producing is increased by electrical stimulation of the splanchnic nerve below the diaphragm.

3. The increased secretion resulting from splanchnic stimulation is independent of the blood-vascular changes simultaneously provoked.

ON SOME ANALOGIES BETWEEN THE PHYSIOLOGICAL EFFECTS OF HIGH TEMPERATURE, LACK OF OXYGEN, AND CERTAIN POISONS.

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DR. LOEB has expressed the opinion in his lectures that high temperatures and other strong stimulations affect the organism in the way that lack of oxygen does, and it was with the intention of testing the correctness of this supposition that the following series of experiments was made.

The theory that strong stimulation acts like the want of oxygen is based upon Hoppe-Seyler's theory of oxidation.[1] In the cell processes of fermentation take place, by means of which highly reducing substances such as nascent hydrogen are formed. These readily unite with atmospheric oxygen, if it be present, forming oxidized products ($H_2 + O_2 = H_2O + O$) and setting free atoms of oxygen. The free atoms of oxygen are then capable of oxidizing other substances (sugar, lactic acid, etc.) at the low temperature of the animal body. But if oxygen is not present, the nascent hydrogen can reduce the substances in the cell and thereby form new products normally not found in the body. The bodies thus formed may be more or less injurious; in fact, they may act as violent poisons.

It is by means of this theory that Dr. Loeb accounts for the difference which he found in the behavior of the heart of Fundulus and Ctenolabrus when deprived of oxygen.[2] The heart of a Fundulus embryo beats normally about 120 times a minute at a certain temperature. If deprived of oxygen, the rate is reduced within less than an hour to twenty beats, but the heart continues beating at this rate for over twelve hours. The embryo heart of Ctenolabrus, on the other hand, if deprived of oxygen, after a few minutes stops almost suddenly without any considerable previous reduction in the rate. Thus the two hearts differ to a remarkable extent. The stopping of the Ctenolabrus heart can hardly be due to lack of energy, for the

[1] HOPPE-SEYLER: Physiologische Chemie, 1878, i, pp. 126 *et seq.*

[2] LOEB, J.: Arch. f. d. ges. Physiol., 1896, lxii, pp. 249–294.

muscles of the body still contract after the heart has ceased to beat. In the case of the Fundulus heart, energy for the slow contractions, which continue many hours without oxygen, probably is furnished by fermentation — the more rapid beat, soon reduced by lack of oxygen, is no doubt the result of oxidation; but the stopping of the Ctenolabrus heart is probably not to be explained by the want of material for fermentation. The most reasonable explanation of the difference in the behavior of the two hearts toward lack of oxygen was furnished by Loeb from experiments on the eggs of the two fish in their early stage of cleavage. When oxygen is lacking, substances are formed in the egg of Ctenolabrus which prevent it from segmenting and which dissolve the cell-walls already formed. In the egg of Fundulus this does not take place, but the egg goes on segmenting for many hours.

It seems probable that such substances are formed in the embryo heart as well as in the egg of Ctenolabrus. The ultimate source of energy of vital action is chemical, but to appear as heart-beat this chemical energy (produced by fermentation and oxidation) must be transformed into molecular energy. To obtain this, certain conditions of protoplasm must exist, and these conditions are normally present in the living heart-muscle. But in the absence of oxygen substances are formed by fermentation which may and most likely do produce grave changes in the protoplasm, — such as changes in surface tension, in osmotic pressure, in the spreading phenomena, — and these changes would prevent the transformation of the liberated chemical energy into that molecular energy required for specific physiological activity.

It may be added that the injurious bodies may be formed in two ways. First, they may be the direct products of fermentation. In this case they would be oxidized, if oxygen were present, and thus rendered harmless; but if oxygen were absent, they would retain their original form, and might produce destructive results. Secondly, these bodies may arise from fermentation secondarily, in consequence of the fermentation products reducing material normally present in the cell and thus making it harmful. This action will never occur if sufficient oxygen is supplied. It may further be added that if Hoppe-Seyler's theory of oxidation is correct, oxygen not only furnishes energy by oxidation but plays an equally important rôle in the synthetical action by which complex bodies are built up for undergoing fermentation.

From these considerations it is evident that death by lack of oxygen may be due to one of two causes: (1) Too little reserve material for fermentation. Here the necessary energy is almost directly dependent upon oxidation, and if the oxygen is not supplied regularly, the death of the organ or organism results almost instantly. (2) To the formation of substances that render the transformation of chemical into molecular energy impossible (Ctenolabrus).

We said there seemed to be reasons for believing that high temperature and strong stimuli in general act as does the lack of oxygen. As by stimulation the processes of fermentation are increased, it is evident that if the stimulation be severe and long continued, the fermentation may be so great that the oxygen supply will be insufficient to oxidize all the reducing substances formed. Hence the same conditions would obtain as in lack of oxygen, and death resulting from high temperature could be referred to one of the above two causes.

It was with the object of testing this theory that, at the request of Dr. Loeb, the following experiments were made. Our investigations comprise (1) The effect of high temperature, (2) of lack of oxygen, and (3) of poisons upon the common infusoria, Paramœcium aurelia.

High Temperature.

The experiments on the effects of high temperature were conducted in the following manner. On a warm stage was placed a glass dish, ten centimetres long, six and a half wide, and three deep, half-filled with water heated to the desired temperature. The Paramœcia were placed in vessels of two different kinds, — the first was a closed dish, holding thirteen to fourteen drops, and admitted no air; the second differed from the first chiefly in that air was admitted. These dishes were so constructed that their contents, having a small bulk and a large area, and being separated from the heated water by thin glass, very speedily acquired the temperature of the surrounding medium. In order to allow for the cooling of the water by the vessel, the water was always heated one degree above the desired temperature, and this was found to balance the cooling quite accurately. The desired temperature of the water was maintained by a regulated gas flame.

(a) **Different temperatures.** — The closed dish (not admitting air) was filled with Paramœcium culture and placed in water of the desired temperature. Column 1 of Table I gives the length of time in minutes required to kill all the infusoria. As the resistance of Paramœcia

varies slightly and as it is impossible always to obtain exactly the same conditions, it is not surprising that the series is not perfectly uniform. From these experiments it follows that the duration of life does not decrease in proportion to the rise in temperature, but decreases more rapidly.

Similar experiments were made in the open dish admitting air. The results are found in Column 2 of Table I. From this table it will be seen that the figures corresponding to 36° and upwards are practically the same in Columns 1 and 2. From 36° downwards, however, they vary widely. To what is the difference due?

We think it is because in the one case no air is present (except that absorbed by the water) while in the other, air is admitted. Although the death of Paramœcia at 28° and 30° is not directly due to the lack of oxygen, still this want is an important factor, as is shown by the fact that Paramœcia placed in the open dish under water having room temperature (15°–20° C.) suffered no change in 17 hours, while Paramœcia placed in the closed vessel (not admitting air), at the same temperature and for the same length of time, were nearly all dead. From these experiments we may draw the conclusion that during the short time that Paramœcia live at a temperature of 36° C., the lack of oxygen makes itself felt very little, compared with the effects produced by the high temperature; below 36° C., however, the lack of oxygen is very apparent.

(b) **Heat and concentration of liquid.** — It has been held by some authors that acclimatization to high temperatures is brought about by loss of water from the organism. Davenport and Castle[1] hold this view, for they say: "We are accordingly led to inquire whether the process of acclimatization to higher temperature may not find its cause in the reduction of the amount of water in the chylema spaces, — the organism becoming in this respect more like the spores in which the quantity of free water is reduced to a minimum." It is true that in some physiological phenomena loss of water has the same effect as a lowering of temperature. Loeb[2] found that the sense of heliotropism of Copepods and Polygordius may be changed from negative to positive by lowering the temperature or by the loss of water caused by increasing the concentration of the sea-water. The positive heliotropism can be changed to negative by increasing

[1] Davenport and Castle: Archiv fur die Entwickelungsmechanik der Organismen, ii, p. 227.

[2] Loeb: Arch. f. d. ges. Physiol., liv, p. 81.

TABLE I.

Duration of life of Paramœcia at different temperature in open and closed dishes and in different solutions.

Temperature.	Duration of Life in Minutes.				
	1 In Paramœcium culture. Closed dish.	2 In Paramœcium culture. Open dish.	3 In $\frac{1}{2}$ % NaCl solution. Closed dish.	4 In $\frac{1}{4}$ % NaCl solution. Closed dish.	5 In $\frac{1}{8}$ % NaCl solution. Closed dish.
17°C. . . .	1560	....	....	....	....
20 . . .	....	....	185	237	....
22 . . .	....	....	87	165	....
24 . . .	....	....	72	82	144
26 . . .	....	....	36	45	146
28 . . .	115	....	24	39	73
30 . . .	100	....	24	25	72
31 . . .	....	459	....	....	....
32 . . .	....	362	11	16	58
33 . . .	....	268	....	....	....
34 . . .	....	154	9	7; 9	26
35 . . .	48	121	9	....	....
36 . . .	31	38; 31	....	11	17
37 . . .	24	22	7	8	....
38 . . .	13	19	....	....	10
39 . . .	....	14	4	6	....
40 . . .	12; 9; 13	12	$2\frac{1}{2}$	....	6
41 . . .	....	....	....	4	....
42 . . .	8	9	....	....	....
43 . . .	....	7	....	....	....
44 . . .	....	$4\frac{1}{2}$	....	....	....

the water in the cell (lowering concentration) or by raising the temperature. In some unpublished researches on Fundulus eggs, prior to the publication of Davenport and Castle, Loeb investigated the

same question which they discuss. He found, however, that by raising the concentration of the sea-water, the eggs were not able to withstand as high a temperature as when in normal sea-water.

It was with this question in mind that we made a series of experiments, employing sodium chloride solutions, having the strength of 0.5, 0.25, and 0.125 per cent. The experiments were performed by carefully placing thirteen drops of the required sodium chloride solution in the closed vessel and adding one drop of Paramœcium culture. The results are found in Columns 3, 4, and 5 of Table I. It is very evident, from these experiments that with the increase of the concentration of the solution in which the Paramœcia live their power of resistance to high temperature is decreased. Even a $\frac{1}{8}$ per cent sodium chloride solution considerably decreased their duration of life when subjected to high temperatures.

(c) **Acids and alkalies.** — We next made a series of experiments with acids and alkalies to ascertain whether the reaction of the fluid in which the infusoria were heated had any effect upon their power of resistance to high temperature. Isotonic solutions of sodium hydrate, hydrochloric acid, and sodium chloride were made. The dish was nearly filled with a certain per cent solution of alkali, acid, or sodium chloride, a drop of Paramœcium culture was added, and then the dish was heated in the water-bath to the required temperature. Table II gives the results obtained in some experiments with sodium hydrate solution, the Paramœcia being heated to 40° C.

TABLE II.

Temperature 40° C. — Open dish (air admitted).

Medium.	Duration of life in minutes.	Average in minutes.	Increase over water in minutes.	% Increase over H_2O.
Culture	22; 22; 25; 23; 22	23	....	....
Distilled Water .	(21; 25); 12; 17; 16; 16; 18; 19; 17; 13; 14; 17	17	....	....
$\frac{1}{4000}$ % NaOH . .	31; 31	31	14	83
$\frac{1}{8000}$ % NaOH . .	(35; 39; 26; 30; 30; 26; 27; 25; 28; 23; 24; 27; 27)	28.2	11.2	66
$\frac{1}{16000}$ % NaOH . .	26; 29	27.5	10.5	62

From this table it is evident that a weak sodium hydrate solution ($\frac{1}{400}$–$\frac{1}{1600}$%) increases the power of resistance of Paramœcia to the destructive effects of high temperature. This result was verified by many subsequent experiments. The result of one lot comprising about sixty experiments is represented graphically in Fig. 1. In

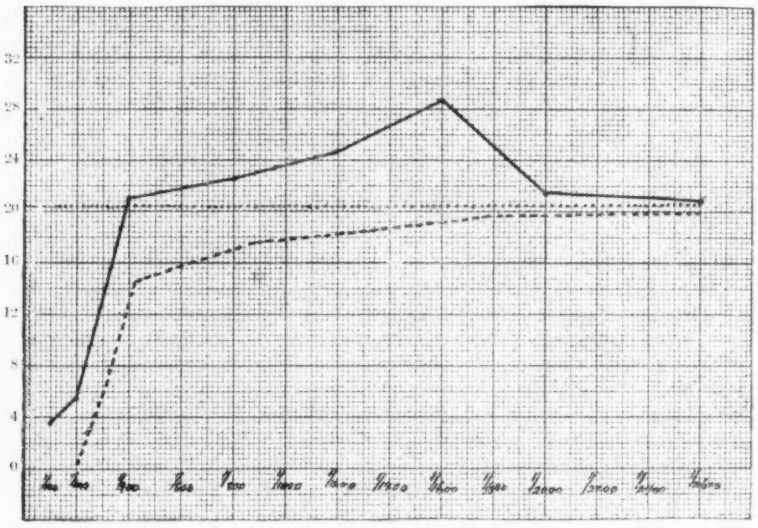

FIGURE 1. Showing duration of life of Paramœcia in acid (broken line) and alkali (unbroken line) solutions, and in distilled water (dotted line), at 40° C. The abscissæ give the per cent of concentration of the acid and alkali solutions; the ordinates give time in minutes.

this figure the degree of concentration of the sodium hydrate and hydrochloric acid solutions is represented by the abscissæ, while the ordinates denote the time in minutes which Paramœcia live in these solutions at 40° C. The full-lined curve represents the length of life at 40° C. in the various concentrations of sodium hydrate, while the broken curve denotes the length of life in hydrochloric acid solutions at the same temperature. The dotted line at 20.5 minutes, running parallel with the axis of abscissa, shows the length of time which Paramœcia live in distilled water at 40° C. As one sees from this figure, the alkali curve between the concentrations of $\frac{1}{400}$% and $\frac{1}{2000}$% is considerably above the straight line which repre-

sents the duration of life in neutral water. In the acid solutions, however, the duration of life (represented by broken curve) is everywhere below this line, showing the acid solution of all concentrations are injurious if they have any effect at all. If the concentration of the alkali solution is greater than $\frac{1}{1000}$% it is decidedly injurious.

The action of a weak alkali solution is still better seen in the following detailed description of two experiments.

Experiment 207.

Temp. 40° *C.* — *Open dish.*

Thirteen drops of distilled water and one drop of Paramœcium culture.
After 10 min. — Movements slower and much rotation.
After 15 min. — Many quiet; others very slow.
After 20 min. — All dead.

Experiment 208

Temp. 40° *C.* — *Open dish.*

Thirteen drops of $\frac{1}{1600}$% NaOH and one drop of Paramœcium culture.
After 10 min. — Movements about normal.
After 15 min. — None quiet yet; moving slowly.
After 20 min. — Only a couple quiet; the rest moving slowly.
After 25 min. — A few more at rest; much like at 15 min. in the water medium.
After 28 min. — All dead.

These experiments show that the conditions after 25 minutes in a $\frac{1}{1600}$% NaOH solution at 40° C. correspond very nearly to that after 15 minutes in distilled water at the same temperature.

Now this beneficial effect of the alkali solution might be due (1) to its osmotic pressure, or (2) to its chemical qualities. If the effect is due to osmotic pressure, an indifferent solution, such as sodium chloride, of the same osmotic value ought to have the same effect. From the molecular weights of sodium chloride (58.5) and of sodium hydrate (40) we find that a $\frac{1}{400}$% NaCl and a $\frac{1}{400}$% NaOH solution have the osmotic pressure of 7.239 and 10.602 mm. respectively, not taking into account dissociation. A $\frac{1}{1600}$% solution of sodium chloride and sodium hydrate have the osmotic value of 1.8 and 2.6 respectively. As the increase in duration of life caused by alkaline solutions is found between these two limits, we are chiefly concerned with these percentages, and here the differences of osmotic pressure for the same percentages of sodium hydrate and sodium chloride are so small as to be safely ignored.

Table III gives the averages of the results obtained in the sodium chloride solutions at 40° C.

TABLE III.

Temp. 40° C.—*Open dish* (*air admitted*).

Percentage of Sodium chloride.	Duration of life in minutes.
0.7	3.5
0.35	8.0
0.1	16.0
0.05	20.5
$\frac{1}{200}$	21.6
$\frac{1}{400}$	19.7
$\frac{1}{1600}$	20.6
0.00	20.0

These experiments prove that a neutral salt solution does not have the power of increasing the resistance of Paramœcia to heat. The peculiar influence of sodium hydrate solution cannot therefore be due to its osmotic pressure, but must be due to chemical influences.

In the same manner in which we tried the effect of alkali we also tried the effect of acid solution. For convenience of comparison we employed hydrochloric acid solutions isotonic with those of sodium hydrate. The osmotic pressure of a $\frac{1}{200}$% hydrochloric acid solution is the same as that of a $\frac{1}{200}$% sodium hydrate solution. A $\frac{1}{1752}$% hydrochloric acid solution corresponds to a $\frac{1}{1600}$% sodium hydrate solution. The results of experiments with hydrochloric acid solutions are represented by the broken curve in Fig. 1. This curve shows that if acids have any effect at all, they have the opposite effect to alkalies, namely, shortening the life of Paramœcia subjected to high temperature. Never does a hydrochloric acid solution increase the power of resistance against high temperature, but even such a weak solution as $\frac{1}{1600}$ per cent decreases the duration of life at 40° C. by about five per cent.

We have thus seen that the peculiar effect of alkali in lengthening the life of Paramœcia exposed to high temperatures (37–40°) is not due to its osmotic pressure. Again we have seen that acids do not possess this peculiar action. From these considerations we may infer that by the action of high temperature a substance is formed in the Paramœcia, which eventually brings about the destruction of the organism. This substance is neutralized by alkali but not by acid. This might lead us still further to suppose that the substance formed is an acid.

Lack of Oxygen.

From the theoretical considerations mentioned at the beginning of this paper we concluded that what took place more rapidly at a high temperature might take place more slowly in lack of oxygen, and that therefore alkali would have the same effect as

in the case of high temperature. To determine this we made the following series of experiments on lack of oxygen. The air was eliminated by the usual method — replacement by hydrogen. For this purpose the well-known Engelmann gas-chamber was employed. The hydrogen was carefully washed in a potassium permanganate solution, a concentrated solution of potassium hydrate, and distilled water. Two, and in some cases three wash-bottles of the alkali were inserted, so that the current of hydrogen passed three times through a layer of potassium hydrate solution of about nine centimetres thickness. All these precautions were taken in order to remove every trace of acid which might have been introduced with the hydrogen. In the Engelmann chamber was placed a slip of glass to which were attached two rubber rings thirteen millimetres in diameter, and one millimetre high. The cells thus formed were capable of holding seven drops of fluid. In one of the cells were placed six drops of water and one drop of washed Paramœcia, while the other cell held six drops of the alkali solution plus one drop of washed Paramœcia. The current was then let in, and the behavior of the animals observed and the time of death noted.

As the alkalinity of the water in the aquarium may be considerable and as the alkalinity and the concentration of the water may vary from day to day, we thought it well to employ "washed" Paramœcia. It is well known that the Paramœcia in the aquarium collect densely around the edge of the dish, two or three millimetres below the surface. It is therefore easy to collect a great number of the infusoria without taking much of the water of the aquarium. They are then placed in tubes of distilled water. Besides the very slight difference of concentration and alkalinity of the cultures used, the varying strength of the hydrogen current has to be taken in consideration; when the current is strong, the time required will of course be much less than when it is weak. From this it becomes evident that individual experiments are not to be compared with each other. For example, it would be incorrect to compare Experiments 248 and 249.

Experiments 248–254 of Table IV give the results of a series which show that in a weak alkali solution the Paramœcia withstand the lack of oxygen for a longer time than in distilled water. The average increase in these four experiments is 116 per cent. Hence the effect of a weak alkali solution in the case of lack of oxygen is the same as in the case of high temperature; in both the resist-

ance of Paramœcia is increased. To show that here also the influence of the sodium hydrate solution is not due to osmotic pressure, we compared the duration of life in water and in a sodium chloride solution. Experiments 387–396 of Table IV give the results of this inquiry. The increase in the sodium chloride solution is very slight compared with the increase found in Exp. 248–254, where sodium hydrate solutions were employed.

TABLE IV.

Duration of life of Paramœcia subjected to lack of oxygen.

No. of Exp.	Duration of life in minutes.					Increase in NaCl over Water.		Increase in NaOH over H_2O or NaCl.	
	In H_2O.	In NaCl.		In NaOH.					
		% Solution.	Time in min.	% Solution.	Time in min.	In min.	In %.	In min.	In %.
248	15	....	....	$\frac{1}{800}$	40	....	....	25	170
249	65	....	....	"	107	....	....	42	70
252	20	....	....	"	42	....	....	22	110
254	33	....	....	"	71	....	....	38	115
387	50	$\frac{1}{400}$	90	....	....	40	80	....	....
388	32	$\frac{1}{100}$	42	....	....	10	30	....	....
389	62	$\frac{1}{200}$	66	....	....	4	$6\frac{1}{2}$	....	....
390	39	$\frac{1}{10}$	42	....	....	3	8	....	....
391	16	$\frac{1}{400}$	13	....	....	−3	−20	....	....
392	32	$\frac{1}{100}$	36	....	....	+4	+32	....	....
393	25	$\frac{1}{10}$	45	....	....	20	80	....	....
394	13	$\frac{1}{700}$	18	....	....	5	40	....	....
395	11	$\frac{1}{700}$	11	....	....	0	0	....	....
396	23	$\frac{1}{700}$	22	....	....	−1	$-4\frac{1}{2}$	....	....
380	....	$\frac{1}{100}$	15	$\frac{1}{400}$	95	....	....	80	540
381	....	$\frac{1}{300}$	28	$\frac{1}{400}$	46	....	....	18	65
382	....	$\frac{1}{1000}$	65	$\frac{1}{2000}$	65	....	....	0	0
383	....	$\frac{1}{80}$	11	$\frac{1}{100}$	22	....	....	11	100
384	....	$\frac{1}{160}$	11	$\frac{1}{200}$	39	....	....	28	255

To show this still more clearly we compared the effect of an alkali and a neutral salt solution side by side, the results of which are given in Exp. 380–384, Table IV. The solution of sodium hydrate and sodium chloride here used were almost isotonic. Neglecting dissociation, the osmotic pressure of

$\frac{1}{300}$ per cent NaCl solution = 9.65 mm.; of $\frac{1}{400}$ per cent NaOH = 10.6 mm.
$\frac{1}{160}$ " " " = 18.09 mm.; of $\frac{1}{200}$ " " = 21.20 mm.
$\frac{1}{80}$ " " " = 36.195 mm.; of $\frac{1}{100}$ " " = 42.40 mm.

These last experiments (380–384) show that the average increase of duration of life in a sodium hydrate solution (above $\frac{1}{2000}$ per cent. over that in the sodium chloride solution is 240 per cent. There is therefore no doubt that in lack of oxygen, as in the case of high temperature, the peculiar effect of alkali solutions is not due to osmotic pressure.

It still remains to show the effects of acid. Table V shows the action of hydrochloric acid compared with water.

TABLE V.

No. of Exp.	Duration of life in minutes.			Increase in HCl.	
	In H_2O.	In HCl Solution.			
		% Solution.	In min.	In min.	In %.
385	33	$\frac{1}{438}$	4	−29	−87
386	21	$\frac{1}{438}$	3	−18	−86
398	30	$\frac{1}{876}$	21	− 9	−30
"	24	$\frac{1}{876}$	15	− 9	−37
"	52	$\frac{1}{1752}$	48	− 4	− 8
"	46	$\frac{1}{1752}$	45	− 1	− 2
"	27	$\frac{1}{3504}$	28	+ 1	+ 4
"	34	$\frac{1}{3504}$	32	− 2	− 6

From this table it will be observed that hydrochloric acid solutions, as in the case of high temperature, act unfavorably upon Paramœcia in case of lack of oxygen.

To show the favorable action of a sodium hydrate solution in detail, we append the notes on Experiment 247.

Experiment 247.

Paramœcium aurelia subject to lack of oxygen.

A number of the Paramœcia are placed in distilled water; the others, in a $\frac{1}{800}$ per cent sodium hydrate solution.

1.15 P.M.	The hydrogen introduced into Engelmann chamber.
1.50 P.M.	In the distilled water, some are entirely quiet; some of them are rotating rapidly, with no progressive movements. A few of the smaller are still swimming. In the $\frac{1}{800}$ % NaOH about 80 per cent are still swimming in a most lively fashion. The rest are rotating. Those in the water appear much swollen, being about twice the size of those in the alkali.
2.05 P.M.	Hardly any life left in the water. About half of those in the alkali are still moving about quite lively; a few absolutely quiet; the remaining are rotating.
2.15 P.M.	No more life left in the water. About half of those in the alkali are still moving quite lively.
2.35 P.M.	About one quarter of those in the alkali swim quite briskly.
2.45 P.M.	In the alkali a few are still moving slowly. *Experiment interrupted.*

Thus we have shown that alkali solutions of from $\frac{1}{200}$ to $\frac{1}{2000}$ per cent increase the resistance power of Paramœcia to both high temperature (40° C.) and lack of oxygen. In this respect we have shown the similarity between the physiological effects of both. We have further shown that this action of the alkali is not due to its osmotic pressure; hence it must be due to its chemical composition. Again it has been proven that acids do not increase the power of resistance to these two agents, but that, if at all active, they have a weakening influence. This might lead us one step further in our theory, namely, that the peculiar action of the alkali in these circumstances is due to the fact that it neutralizes a substance, perhaps an acid, formed by strong stimulation and during lack of oxygen.

Poisons.

Through the observations of Claude Bernard it has been proven that if an animal is killed by potassium cyanide the blood in the veins remains arterial, and Geppert proved that potassium cyanide renders the tissues unable to take up oxygen. Such a manner of death is therefore comparable to that brought about directly by lack of oxygen. If this is correct, we ought to be able to demonstrate in the case of poisoning by potassium cyanide, the peculiar action of alkali that we found in the case of lack of oxygen. With this in mind, we made the following series of experiments.

TABLE VI.

One per cent potassium cyanide: effects of alkali.
10 drops of the liquids H_2O, NaOH, HCl, or NaCl +
1 drop of washed Paramœcia plus 1 drop of 1.0% KCN.

No. of Exp.	Duration of life in minutes			Increase in min. in NaOH over		Increase in % in NaOH over		Average increase in NaOH
	In H_2O	In NaCl $\frac{1}{200}$%	In NaOH $\frac{1}{200}$%	H_2O	NaCl	H_2O	NaCl	
256	28	11	30	2	17	7	160	Average
257	36	36	61	25	25	70	70	increase in
258	8	7	28	20	21	250	280	min. over
259	12	14	30	18	16	150	115	H_2O = 13
260	8	15	26	18	11	225	75	NaCl = 12.6
261	12	12	29	17	17	140	140	In per cent over
262	9	19	23	14	4	155	22	H_2O = 118%
263	7	8	13	6	5	90	80	NaCl = 103%
264	11	11	16	5	5	45	45	
265	11	11	16	5	5	45	45	
		0.1%	$\frac{1}{1000}$%					Av. increase
282	5	5	21	16	16	320	320	in min. over
283	7	6	21	14	15	200	250	H_2O = 11.3
284	8	5	17	9	12	115	240	NaCl = 15.0
285	8	5	21	13	16	165	320	In per cent
286	15	7	23	8	16	55	230	H_2O = 162%
287	7	. .	15	8	. .	115	. .	NaCl = 272%

In a small dish, eight or ten drops of distilled water were placed, to which was added one drop of Paramœcia and one drop of the chemical agent to be tested. In a second dish, the water was replaced by a solution of sodium chloride, sodium hydrate, or hydrochloric acid, while the rest remained the same. In most of the experiments three trials were made at once, one in water, one in an

TABLE VII.

One per cent potassium cyanide; effects of acid.
Conditions the same as in Table VI.

No. of Exp.	Duration of life in minutes			Increase in min. in NaOH over		Increase in % in NaOH over		Average increase in NaCH
	In H_2O	In HCl $\frac{1}{138}$%	In NaOH $\frac{1}{200}$%	H_2O	HCl	H_2O	HCl	
267	12	10	18	6	8	50	80	
268	6	4	11	5	7	85	175	
		$\frac{1}{876}$%						
269	5	3	7	2	4	40	135	
270	9	8	12	3	4	35	50	
		$\frac{1}{1752}$%	$\frac{1}{100}$%					Av. increase in
272	5	5	8	3	3	60	60	min. over
273	9	8	13	4	5	45	63	H_2O = 4, or 58%
274	7	7	12	5	5	70	70	HCl = 4.4 or 64%
			$\frac{1}{1200}$%					
275	7	6	7	0	1	0	1½	Av. increase in
276	8	8	11	3	3	36	36	min. over
277	10	9	15	5	6	50	65	H_2O = 2.7 or 29%
								HCl = 3.4 or 34%

If 0.7 per cent sodium chloride is used, death occurs almost instantly. If 0.025 per-cent sodium hydrate is used, death occurs in about one minute. The one per cent potassium cyanide has a strong alkaline reaction.

alkali solution, and the third in a sodium chloride solution. The three tests were made side by side for ready comparison, and at exactly the same time.

We first made experiments with potassium cyanide, the results of which are embodied in Tables VI and VII.

TABLE VIII.

One tenth per cent sulphate of Atropine; effects of alkali and acid.

Eight drops of H_2O, NaOH, NaCl, or HCl solutions plus one drop of washed Paramœcia plus two drops of 0.1 per cent Atropine.

No. of Exp.	Duration of life in minutes			Increase in min. in NaOH over		Increase in % in NaOH over		Average increase in NaOH
	In H_2O	NaCl $\frac{1}{200}$ %	NaOH $\frac{1}{100}$ %	H_2O	NaCl	H_2O	NaCl	
288	3	3	6	3	3	100	100	Over H_2O = 5 min. or 124%
289	3	4	10	7	6	235	150	
290	14	5	17	3	12	21	240	Over NaCl = 7 min. or 157%
291	5	5	12	7	7	140	140	
		$\frac{1}{400}$ %	$\frac{1}{200}$ %					Over H_2O = 22 min. or 418%
292	8	6	26	18	20	225	330	
293	4	6	32	28	26	700	430	Over NaCl = 20 min. or 307%
294	6	10	26	20	16	330	160	
		$\frac{1}{800}$ %	$\frac{1}{400}$ %					
295	9	4	11	2	7	22	175	Over H_2O = 3½ min. or 76%
296	4	10	13	9	3	225	30	
297	12	12	22	10	10	85	85	Over NaCl = 3 min. or 65%
298	27	28	20	−7	−8	−25	−30	
			$\frac{1}{1200}$ %					
299	21	21	22	1	1	5	5	Over H_2O = 1 min. or 6% ?
300	13	13	14	1	1	7	7	
		$\frac{1}{1600}$ %	$\frac{1}{800}$ % HCl					
301	2	2	3					
302	4	4	4					
303	5	3	7					
304	25	25	25					
305	4	4	4					
			$\frac{1}{1.8}$ % HCl					
306	10		4					
307	11		1					

The 0.1 per cent atropine has no effect upon litmus. A strong solution, however, is decidedly alkaline.

TABLE IX.

Sulphate of strychnine 0.01 per cent; effects of alkali.

Eight drops of H_2O, NaCl, NaOH, plus one drop of washed Paramœcia plus one drop of 0.01 per cent sulphate of strychnine.

No. of Exp.	Duration of life in minutes			Increase in NaOH over H_2O		Average increase of NaOH over H_2O or NaCl
	In H_2O	In NaCl $\frac{1}{100}$ %	In NaOH $\frac{1}{100}$ %	In min.	In %	
308	5	5	0		$-\infty$	
		$\frac{1}{400}$ %	$\frac{1}{200}$ %			
310	11	11	4	−7	−65	
311	5	3	1	−4	−80	−73%
312	5	6	1	−4	−80	
313	10	11	3	−7	−70	
		$\frac{1}{1600}$ %	$\frac{1}{1200}$ %			
317	6	4	4	−2	−35	
318	6	6	4	−2	−35	−40%
319	4	4	2	−2	−50	
		$\frac{1}{3200}$ %	$\frac{1}{2000}$ %			
322	5	5	4	−1	−20	
323	5	5	5	−0	−0	−9%
324	3	5	3	−0	−0	
325	7	9	6	−1	−15	

From these tables it is apparent that a weak alkali increases the power of resistance against potassium cyanide while a neutral or acid solution does not possess this power. In fact, while the extreme weak alkali solution of $\frac{1}{2000}$ per cent still increases the time twenty-three per cent, a solution of $\frac{1}{1752}$ per cent hydrochloric acid either

shortens the time or has no effect whatever. That the action of the sodium hydrate is not due to the osmotic pressure is shown in Experiments 256–265, where the difference of pressure between the sodium chloride and sodium hydrate solutions is but seven millimetres.

From observations made by Rossbach it seems likely that alkaloids may also produce death by rendering the tissues unable to take up oxygen. This led us to test the action of acids and alkalies in case of poisoning by atropine, strychnine, etc. The results obtained with atropine poisoning are embodied in Table VIII.

In the experiments with atropine, the beneficial action of sodium hydrate is still better shown than in the case of potassium cyanide. When it is remembered that the alkaloid is already alkaline in its reaction, it is evident that this action of sodium hydrate is not due to the neutralization of the alkaloid. That this peculiar action of alkali is not due to a general stimulating effect or the neutralization of the poison, the following experiments on strychnine (308–329) prove conclusively.

TABLE X.

Sulphate of strychnine 0.01 per cent: effects of acid. Conditions the same as in Table IX.

No. of Exp.	Duration of life in minutes.			Increase in NaCl.		Average Increase.
	In H_2O.	In NaCl 0.1 %.	In HCl $\frac{1}{438}$ %.	In min.	In %.	
326	5	18	0.0	13	260	Of 0.1 % NaCl over H_2O = 8.2 min. or 144 %.
			$\frac{1}{1752}$ %			
327	8	16	7	8	100	
328	11	18	11	7	160	
329	9	14	9	5	55	

Sulphate of strychnine 0.01 per cent has no effect upon the litmus, but a stronger solution is decidedly acid in reaction. If 0.7 per cent sodium chloride is used, the Paramœcia perish in one or two minutes.

From these experiments it is apparent that a sodium hydrate solution, instead of exercising a beneficial influence, is highly

destructive. This is so radically different from the trend of all the preceding experiments, that I doubted the purity of the solution. But on testing, I found that the Paramœcia in a $\frac{1}{200}$ per cent sodium hydrate solution lived for more than three hours.

Considering that the strychnine is acid in reaction, while the potassium cyanide and atropine solutions are alkaline, we would *a priori* expect that an alkali ought to be more beneficial when added to a strychnine solution than to the others. Instead we found the opposite true. Most likely the chemical action of the strychnine in the cell is different from that of potassium cyanide or atropine. This corresponds with the difference in the physiological effects of these poisons. But even in some alkaloids having an alkaline reaction, the addition of a weak alkali acts unfavorably, as is shown in Experiments 353–358.

TABLE XI. — VERATRINE.

Thirty-five mg. of veratrine boiled in 10 c.c. of water and filtered.

Eight drops of H_2O, HCl, NaCl, or NaOH solutions plus one drop of washed Paramœcia plus two drops of veratrine.

No. of Exp.	Duration of life in minutes.		Decrease in NaOH (in min.)
	In H_2O.	In NaOH	
		$\frac{1}{200}$ %	
353	26	2	24
354	40	2	38
355	28	$1\frac{1}{2}$	$26\frac{1}{2}$
		$\frac{1}{400}$ %	
356	41	10	31
357	18	15	3
		$\frac{1}{1752}$ % HCl	
358	36	34	

If a $\frac{1}{1755}$ % HCl solution is used, the Paramœcia are destroyed almost instantly. The reaction of this veratrine solution is slightly alkaline.

These experiments show that in case of veratrine poisioning, alkali does not increase the duration of life of Paramœcia, but shortens it considerably. Experiments on other poisons are in progress.

When we review the poisons thus far experimented upon we find that their chemical reaction, whether acid or alkaline, does not determine whether the addition of an alkali renders the organism more resistant to its actions or not. The effects of strychnine, although it has an acid reaction, are not counteracted by alkalies. Although potassium cyanide and atropine are alkaline, yet their effects are reduced very markedly by sodium hydrate solutions. It may be further remarked that in no case of poisoning has the addition of an acid increased the resistance of Paramœcia against the poison. As in the case of heat and lack of oxygen, acid, if active at all, decreases the duration of life of the infusoria.

Theoretical Remarks.

Some authors[1] have attributed death caused by a temperature between 35° and 40° C. to a process of coagulation. Others suppose that coagulation is not the direct cause of death, but that other besides physical causes may be present. Among those who sought to attribute death produced by a temperature of 35° to 40° C. to chemical, and not only to physical causes, may be mentioned Sachs, Engelmann, and Rossbach.

Finding that the life of plants is destroyed by a temperature much below that of coagulation, Sachs[2] says, "that the coagulation of the protoplasm is not the only point which determines death at high temperature. . . . So long as outside influences do not exceed a certain amount of force, they are not able to subdue the molecular force [in a cell] which binds together the inner organic structure. But if a force, *e. g.* heat, reaches an intensity which exceeds the molecular force, the molecules are torn from their normal position, and the inner structure which serves as the carrier of life is destroyed without any real changes in the outward form." Roth[3] extended our knowledge of death at high temperature by showing that alkali solutions are able to revive cilia in heat rigor. Two years later Engelmann

[1] See Kühne: Untersuchungen über das Protoplasma und die Contractilität, Leipzig, 1864.

[2] Sachs, J.: Flora, 1864, xlvii, pp. 5, 24, 33, 65.

[3] Roth, M.: Virchow's Archiv f. pathol. Anatomie, 1866, xxxvii, p. 184.

confirmed this fact. Speaking of this relation between heat and alkalies he says: "It seems to me that the increase of mechanical activity of the cell and the acceleration of the movements produced by heating, are mostly due to the increase in the physiological metabolism (Stoffumsatz). According to our view, in the cells whose movements are increased by heating, the formation of acids is increased. This increased acid formation can, in part, be the cause of the rigor which sets in at 40° C."[1] Rossbach[2] thinks that we cannot in general consider coagulation as the cause of the cessation of movements at high temperature.

Our conception agrees somewhat with that of Engelmann, as do also, to a certain extent, the facts found.

Our object, as stated in the introduction, was to endeavor to establish the similarity between the effects of high temperature and lack of oxygen. Heat, below the point of coagulation, by increasing fermentation, sets free chemical energy, which can be transformed into molecular energy, appearing in such vital phenomena as ciliary motion, contracting of vacuoles, etc.

But we have above supposed that if the stimulation be very strong and of long duration, the oxidation would be insufficient to oxidize all the reducing products formed by fermentation, and that these products would act upon the contents of the cell in such a way as to render the change of chemical into molecular energy impossible, thereby producing death. *A priori*, it hardly seems likely that Paramœcia in an indifferent solution should die in from thirty to sixty minutes because of lack of energy caused by want of oxygen. But if destructive substances be formed, it is easily seen how a sudden death might occur, when we bear in mind the rapid death produced by potassium cyanide and alkaloids. Now from the fact that alkali increases the resistance of Paramœcia to both lack of oxygen and high temperatures, we surmise that heat, below the point of coagulation, brings about death in the same manner as lack of oxygen. It may be supposed that the harmful substances, formed by metabolism at high temperature and in lack of oxygen can in some way be rendered inactive, and in this we believe lies the beneficial action of alkali. In some way or other, either by neutralizing their effects or by attacking the substances

[1] Engelmann, T. W.: Ueber die Flimmerbewegung, Leipzig, 1868. See also Engelmann: Centralblatt für die medicinische Wissenchaften, 1867, p. 657.

[2] Rossbach, J.: Arbeiten aus der zoologischen und zootomischen Institut in Würzburg, 1874, i. p. 9

themselves, alkali delays death. Seeing they are rendered harmless by alkalies and not by acids, it seems possible that these products formed by fermentation are acids. This also agrees with the experiments of Gaule,[1] who made the important discovery that a weak sodium hydrate solution (1 : 20,000) revived the excised frog heart come to rest in a saline solution. He showed also that such an alkaline solution after being repeatedly driven through the heart underwent a loss in alkalinity, due, no doubt, to an acid formed in the heart muscle.

As to the action of potassium cyanide and atropine, it is to be borne in mind that we do not regard these substances as stimuli, as has been well shown by Rossbach in the paper above referred to. This author also pointed out the similarity of action between lack of oxygen (by hydrogen atmosphere) and alkaloids, and he thinks that we may consider the cessation of oxidation of the protoplasm as the ultimate action of alkaloids. Supporting this view, Dr. Budgett[2] has shown the striking resemblance between the morphological changes produced in case of lack of oxygen and by alkaloid poisoning upon Paramœcia.

If it is true that these poisons kill by diminishing the power of oxidation, while fermentation continues, reducing substances will be formed, and these substances will have the effects already described. Alkali will therefore have the same influence as in lack of oxygen and high temperature. It may be added that there remains the possibility of the sodium hydrate having some chemical reaction upon the potassium cyanide and atropine, but as the percentage of alkali used is so very small, this becomes highly improbable.

SUMMARY.

Alkalies in very small percentage ($\frac{1}{4000}$ – $\frac{1}{20000}$ per cent) increase the resistance of Paramœcia to heat (36–40°C.), to the lack of oxygen, and to the destructive action of potassium cyanide and atropine. Acids (hydrochloric), on the other hand, never increase, but when at all active, always decrease the power of resistance to the agents named.

These facts we try to explain in that the lack of oxygen (produced by hydrogen atmosphere or by poisons preventing oxidation) and

[1] GAULE, J.: Archiv fur Physiologie, 1878, p. 291.

[2] BUDGETT: This journal, 1898, i. p. 98.

high temperature cause the formation of certain substances which render the transformation of chemical into molecular energy impossible, thereby producing death. These substances are in some way antagonized by alkalies, but not by acids.

I take this opportunity to express my thanks to Dr. Loeb for aid kindly given in the execution of this work.